"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF MEBENDAZOLE CHEWABLE TABLETS BY RP-HPLC"

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted
By

Reg No: 261230957

DEPARTMENT OF PHARMACEUTICAL ANALYSIS

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

APRIL 2014

"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF MEBENDAZOLE CHEWABLE TABLETS BY RP-HPLC"

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted By

M. SANKAR., B. Pharamcy

(Reg No: 261230957)

Under the guidance of

Prof. Dr.S.Vadivelan, M.Pharm., Ph.D.,

DEPARTMENT OF PHARMACEUTICS

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

APRIL 2014

Prof.Dr.S.Vadivelan, M.Pharm., Ph.D.,

Associate Professor,

Department of Pharmaceutical Analysis,

Edayathangudy.G.S.Pillay College of Pharmacy,

Nagapattinam – 611 002.

CERTIFICATE

This is to certify that the dissertation entitled **"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF MEBENDAZOLE CHEWABLE TABLETS BY RP-HPLC"** submitted by **M. SANKAR** (Reg No: 261230957) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in the Department of Pharmaceutical Analysis, Edayathangudy.G.S Pillay College of Pharmacy during the academic year 2013-2014.

Place: Nagapattinam **Dr.S.Vadivelan, M.Pharm.,Ph.D.,**

Date:

Prof.Dr.D.Babu Ananth,M.Pharm., Ph.D.,

Principal,

Edayathangudy.G.S.Pillay College of Pharmacy,

Nagapattinam – 611 002.

CERTIFICATE

This is to certify that the dissertation **"Analytical Method Development and Validation for Quantitative Estimation of Mebendazole Chewable tablets by RP-HPLC"** submitted by **M. SANKAR** (Reg No: 261230957) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under the guidance of **Dr.S.Vadivelan, M.Pharm.,Ph.D.,** Associate Professor, Department of Pharmaceutical Analysis, Edayathangudy.G.S Pillay College of Pharmacy during the academic year 2013-2014.

Place: Nagapattinam **Prof.Dr.D.Babu Ananth,M.Pharm., Ph.D.,**

Date:

ACKNOWLEDGEMENT

I would like to express profound gratitude to **Chevalier Thiru.G.S.Pillay,** Chairman, E.G.S.Pillay College of Pharmacy, and **Thiru. S.Paramesvaran, M.Com., FCCA.,**Secretary, E.G.S.Pillay College of Pharmacy.

I express my sincere and deep sense of gratitude to my guide **Dr.S.Vadivelan, M.Pharm.,Ph.D.,**Department of Pharmaceutical Analysis, E.G.S.Pillay College of Pharmacy, for his invaluable and extreme support, encouragement, and co-operation throughout the course of my work.

It is my privilege to express my heartfelt thanks to **Prof. Dr.D.Babu Ananth, M.Pharm, Ph.D.,** Principal, E.G.S.Pillay College of Pharmacy, for providing me all facilities and encouragement throughout the research work.

I express my sincere gratitude to **Prof. Dr.M.Murugan, M.Pharm., Ph.D.**,Director cum Professor, Head, Department of Pharmaceutics. E.G.S.Pillay College of Pharmacy, for his encouragement throughout the course of my work.

I wish to express my great thanks to **Dr.P.Dheen Kumar, M.Pharm., Ph.D.,** Associate Professor , Department of Pharmaceutical Analysis, E.G.S.Pillay College of Pharmacy, for his support and valuable guidance during my project work.

I would like to extend my thanks to all the **Teaching Staff**and **Non-Teaching Staff**, who are all supported me for the successful completion of my project work.

Last but not least, I express my deep sense of gratitude to my parents, family members and friends for their constant valuable blessings and kindness.

INDEX

KEMWELL

Keeping You Competitive

Kemwell Biopharma Pvt.Ltd
34th KM, Tumkur Road,
T.Begur, Nelamangala Taluk,
Bangalore - 562 123. INDIA
Phone: +91-80-27730346-48
Fax : +91-80-27733273
kemwell@kemwellpharma.com
www.kemwellbiopharma.com

TO WHOM SO EVER IT MAY CONCERN

Date: - 10/02/2014

This is to certify that **Mr. Sankar. M** a student from Edayathangudy G.S.Pillay College of Pharmacy has successfully completed his project titled **"Analytical Method Development and Validation for Quantitative Estimation of Mebendazole Chewable Tablets 500mg by RP-HPLC"** in our laboratory with reference to the partial fulfilment of the requirement of the Master of Pharmacy Course of The Tamilnadu, Dr.MGR Medical university, Chennai.

Best regards,
Head of the department (Analytical Development).

For KEMWELL BIOPHARMA Pvt. Ltd.
Bangalore, Karnataka.

LIST OF ABBREVATION

Abs	Absorbance
A.R	Analytical Reagent
℃	Degree centigrade
Conc.	Concentration
e.g	Example
EC	European Committee
FDA	Food and Drug Administration
Gm	Gram
GC	Gas Chromatography
Hrs	Hours
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
ICH	International Conference of harmonization
LOD	Limit of Detection
LOQ	Limit of Quantification
LC-MS	Liquid chromatography-Mass Spectroscopy
M	Molarity
Mg	Milli gram
min	Minutes
mL	Milli liter
N	Normality

NLT	Not Less Than
nm	Nano meter
NMT	Not More Than
%	Percentage
UV	Ultra violet
QC	Quality Control
RSD	Relative Standard Deviation
RT	Retention time
SD	Standard deviation
S.No	Serial Number
µm	Micro meters
µ	Microns
µl	Micro liter
USP	United States of pharmacopoeia
UV	Ultra violet
V	Volume
Wt.	Weight
WHO	World Health Organization

LIST OF FIGURES

INTRODUCTION

The federal food, drug, and cosmetic act defines a drug as follows:

The term "drug" means (a) articles recognized in the official United states pharmacopoeia, official homoeopathic pharmacopoeia of the united states, or official national formulary.; and (b) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man and other animals; and (c) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (d) articles intended for use as a component of any articles specified in (a), (b) or (c).[1]

Pharmaceutical analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk drug and pharmaceutical preparations.[2]

In general terms, pharmaceutical analysis comprises those procedures necessary to determine the "identity, strength, quality, and purity" of such articles. For practical reasons, however, it is proper to broaden the scope of this definition to include the analysis of raw materials. Analytical chemists in the pharmaceutical industry, as well as in those chemical industries that produce pharmaceutical raw materials, must perform such analyses. The raw materials employed in the

production of modern drugs and the intermediates appearing during research, development and synthesis, involve thousands of diverse organic compounds. The pharmaceutical analyst must, therefore, have a firm grounding in basic organic analysis in addition to special skill in the quality evaluation of drug products.

Laboratories requiring pharmaceutical analysis may be classified as follow:

1. government regulatory agencies,
2. manufacturers of drugs,
3. manufacturers of raw materials for drugs,
4. university and other non-commercial research centres,
5. Consulting laboratories.

The principal federal agencies that regulate the drug industry are the food and drug administration (F.D.A) and the U.S. public health service. The latter is responsible for biological products such as vaccines and antitoxins that are tested largely by biological rather than chemical methods.

The food and drug administration, an agency of the department of health, education and welfare, administers and enforces the federal food, drug, and cosmetic Act. It is important that everyone concerned with medicinal products, whether in research, production, control, prescribing, or dispensing, understand this law, the reasons the congress had for its enactment, and the machinery provided its enforcement. This is especially so for pharmaceutical analysts, since

otherwise they can have no adequate appreciation of their front-line role as guardians of the public health[1].

In a modern industrialized society the analytical chemist has a very important role to play. Thus most manufacturing industries rely upon both qualitative and quantitative chemical analysis to ensure that the raw materials used meet certain specifications, and also to check the quality of the final product. The examination of raw materials is carried out to ensure that there are no unusual substances present which might be deleterious to the manufacturing process or appear as a harmful impurity in the final product. Further, since the value of the raw material may be governed by the amount of the required ingredient which it contains, a quantitative analysis is performed to establish the proportion of the essential component: this procedure is often referred to as assaying. The final manufactured product is subject to quality control to ensure that its essential components are present within a pre-determined range of composition, whilst impurities do not exceed certain specified limits.

Quality Assurance plays a central role in determining the safety and efficacy of medicines. Highly specific and sensitive and analytical techniques hold the key to the design, development, standardization and quality control of medicinal products. They are equally important in pharmacokinetics and in drug metabolism studies, both of which are fundamental to the assessment of bioavailability and the duration of clinical response. However modern concepts of

quality differs and concerned not only with chemical purity, but also with those other characterstics of pharmaceutical materials which may influence safety, efficacy, formulation and processing of medicines.[3]

TYPES OF ANALYSIS:

Important factors which must be taken into account when selecting an appropriate method of analysis include

(a) The nature of the information which is sought,

(b) The size of sample available and the proportion of the constituent to be determined, and

(c) The purpose for which the analytical data are required.

The nature of the information sought may involve requirement for very detailed data, or alternatively, results of a general character may suffice with respect to the information which is furnished.

Different types of chemical analysis may be classified as follows:

Proximate analysis, in which the amount of each element in a sample is determined with no concern as to the actual compounds present;

Partial analysis, which deals with the determination of selected constituents in the sample;

Trace constituent analysis, a specialized instance of partial analysis in which we are concerned with the determination of specified components present in minute quantity;

Complete analysis, when the proportion of each component of the sample is determined.

On the basis of sample size, analytical methods are often classified as:

1. Macro, the analysis of quantities of 0.1g or more;
2. Meso(semi micro),dealing with quantities ranging from 10^{-2}g to 10^{-1}g ;
3. Micro, for quantities in the range 10^{-3} g to 10^{-2} g;
4. Sub micro, for samples in the range 10^{-4} g to 10^{-3} g;
5. Ultra micro, for quantities below 10^{-4} g.

The term 'semi micro' given as an alternative name for classification (2) is not very apt, referring as it does to samples larger than micro.

A major constituent is one accounting for 1-100 percent of the sample under investigation; a minor constituent is one present in the range 0.01-1percent; a trace constituent is one present at a concentration of less than 0.01percent. with the development of increasingly sophisticated analytical techniques it has become possible to determine substances present in quantities much lower than the 0.01% upper level set for trace constituents. It is therefore necessary to make further subdivisions: trace corresponds to 102-104 µg per gram, or 102- 104 parts per

million (ppm), micro trace to 102-10^{-1}pg per gram, (10^{-4}-10^{-7} ppm), nanotrace to 102-10^{-1}fm per gram (10^{-7}-10^{-10}ppm).

When the sample weight is small (0.1-1.0mg), the determination of a trace component at the 0.01 percent level may be referred to as sub trace analysis. If the trace component is at the microtrace level, the analysis is termed submicrotrace. With a still smaller sample (not larger than 0.1mg) the determination of a component at the trace level is referred to as ultra trace analysis, whilst with a component at the microtrace level, the analysis is referred to as ultra-microtrace.

The purpose for which the analytical data are required may perhaps be related to process control and quality control. In such circumstance the objective is checking that raw materials and finished products conform to specification, and it may also be concerned with monitoring various stages in a manufacturing process. For this kind of determination methods must be employed which are quick and which can be readily adapted for routine work: in this area instrumental methods have an important role to play, and in certain cases may lend themselves to automation. On the other hand, the problem may be one which requires detailed consideration and which may be regarded as being more in the nature of a research topic.

COMMON TECHNIQUES:

The main techniques employed in quantitative analysis are based upon:

- The quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained;
- Appropriate electrical measurements(e.g. potentiometry);
- The measurement of certain optical properties (e.g. absorption spectra).

In some cases, a combination of optical or electrical measurements and quantitative chemical reaction (e.g. amperometric titration) may be used.

The quantitative execution of chemical reactions is the basis of the traditional or 'classical' methods of chemical analysis: gravimetry, titrimetry, and volumetry. In gravimetric analysis the substance being determined is converted into an insoluble precipitate which is collected and weighed, or in the special case of electrogravimetry electrolysis is carried out and the material deposited on one of the electrodes is weighed.

In titrimetric analysis the substance to be determined is allowed to react with an appropriate reagent added as a standard solution, and the volume of solution needed for complete reaction is determined.

The common types of reaction which are used in titrimetry are:

(a) Neutralization (acid-base) reactions;

(b) Complex-forming reactions;

(c) Precipitation reactions;

(d) Oxidation-reduction reactions.

Volumetry is concerned with measuring the volume of gas evolved or absorbed in a chemical reaction.

Electrical methods of analysis (apart from electrogravimetry referred to above) involve the measurement of current, voltage or resistance in relation to the concentration of a certain species in solution.

Techniques which can be included under this general heading are:

1. Voltammetry (measurement of current at a micro-electrode at a specified voltage);
2. Coulometry (measurement of current and time needed to complete an electrochemical reaction or to generate sufficient material to react completely with a specified reagent);
3. Potentiometry (measurement of the potential of an electrode in equilibrium with an ion to be determined)
4. Conductimetry (measurement of the electrical conductivity of a solution).

Optical methods of analysis are dependent either upon

- Measurement of the amount of radiant energy of a particular wavelength absorbed by the sample, or
- The emission of radiant energy and measurement of the amount of energy of a particular wavelength emitted.

Absorption methods are usually classified according to the wavelength involved as

(a) Visible spectrophotometry (colorimetry),

(b) Ultraviolet spectrophotometry, and

(c) Infrared spectrophotometry.

Atomic absorption spectroscopy involves atomizing the specimen, often by spraying a solution of the sample into a flame, and then studying the absorption of radiation from an electric lamp producing the spectrum of the element to be determined.

Although not strictly absorption methods in the sense in which the term is usually employed, turbidimetric and nephelometric methods which involve measuring the amount of light stopped or scattered by a suspension should also be mentioned at this point.

Emission methods involve subjecting the sample to heat or electrical treatment so that atoms are raised to excited states causing them to emit energy: it is the intensity of this emitted energy which is measured.[2, 4]

Table: 1. List of Common Solvents used in UV Spectroscopy[5]

Solvents	**Cut off wavelength(nm)**
Acetonitrile	190
Water	191
Cyclohexane	195
Hexane	201
Methanol	203
Ethanol	204
Ether	215
Methylene dichloride	220
Chloroform	237
Carbon tetrachloride	257

The common excitation techniques are:

Emission spectroscopy, where the sample is subjected to an electric arc or spark plasma and the light emitted (which may extend into the ultraviolet region) is examined;

Flame photometry, in which a solution of the sample is injected into a flame,which evaporates the solvent,sublimates and atomizes the metal. Light is emitted at characteristic wavelength for each metal.

Fluorimetry, in which a suitable substance in solution (commonly a metalfluorescent reagent complex) is excited by irradiation with visible or ultraviolet radiation.

Chromatography is a separation process employed for the separation of mixtures of substances. It is widely used for the identification of the components of mixtures, but, it is often possible to use the procedure to make quantitative determinations, particularly when using gas chromatography (GC) and high performance liquid chromatography (HPLC).

INSTRUMENTAL METHODS:

The methods dependent upon measurement of an electrical property, and those based upon determination of the extent to which radiation is absorbed or upon assessment of the intensity of emitted radiation, all require the use of a suitable instrument ,e.g. polarograph, spectrophotometer, etc., and in consequence

such methods are referred to as 'instrumental methods'. Instrumental methods are usually much faster than purely chemical procedures, they are normally applicable at concentrations far too small to be amenable to determination by classical methods, and they find wide application in industry .in most cases a microcomputer can be interfaced to the instrument so that absorption curves, polarograms, titration curves, etc., can be plotted automatically, and in fact, by the incorporation of appropriate servo-mechanisms, the whole analytical process may, in suitable cases, completely automated.

Despite the advantages possessed by instrumental methods in many directions, their widespread adoption has not rendered the purely chemical or 'classical' methods obsolete; the situation is influenced by three main factors.

The apparatus required for classical procedures is cheap and readily available in all laboratories, but many instrument are expensive and their use will only be justified if numerous samples have to be analyzed, or when dealing with the determination of substances present in minute quantities (trace,subtrace or ultratrace analysis).With instrumental methods it is necessary to carry out a calibration operation using a sample of material of know composition as reference substance.

Whilst an instrumental method is ideally suited to the performance of a large number of routine determinations, for an occasional, non-routine, analysis it

is often simpler to use a classical method than to go to the trouble of preparing requisite standards and carrying out the calibration of an instrument.

Clearly, instrumental and classical methods must be regarded as supplementing each other.

OTHER TECHNIQUES:

In addition to the main general methods of analysis outlined above there are also certain specialized techniques which applied in special circumstances .Among these are X-ray methods, methods based upon the measurement of radioactivity, mass spectrometry, the so-called kinetic methods, and thermal methods.

X-ray methods:

When high-speed electrons collide with a solid target (which can be the material under investigation), X-rays are produced. These are often referred to as primary X-rays, and arise because the electron beam may displace an electron from the inner electron shells of an atom in the target ,and the electron lost is then replaced by one from an outer shell; in this process energy is emitted as X-rays. In the resultant X-ray emission it is possible to identify certain emission peaks which are characteristic of elements contained in the target. The wavelengths of the peaks can be related to the atomic number of the elements producing them, and thus provide a means of identifying elements present in the target sample. Further, under controlled conditions, the intensity of the peaks can be used to determine the

amounts of the various elements present. This is the basis of electron probe microanalysis, in which a small target area of the sample is pinpointed for examination .This has important applications in metallurgical research, in the examination of geological samples, and in determining whether biological materials contain metallic elements.

When a beam of primary X-rays of short wavelength strikes a solid target, by a similar mechanism to that described above, the target material will emit X-rays at wavelengths characteristic of the atoms involved: the resultant emission is termed secondary or fluorescence radiation. The sample area can be large, and quantitative results obtain by examining the peak heights of the fluorescence radiation can be taken as indicative of sample composition. X-ray fluorescence analysis is a rapid process which finds application in metallurgical laboratories, in the processing of metallic ores, and in the cement industry.

Crystalline material will diffract a beam of X-rays, and X-ray powder diffractometry can be used to identify components of mixtures. These X-ray procedures are examples of non-destructive methods of analysis.

Radioactivity:

Methods based on the measurement of radioactivity belong to the realm of radiochemistry and may involve measurement of the intensity of the radiation from a naturally radioactive material; measurement of induced radioactivity

arising from exposure of the sample under investigation to a neutron source (activation analysis); or the application of what is known as the isotope dilution technique.

Typical applications of such methods are the determination of trace elements in

(a) The investigation of pollution problems;

(b) The examination of geological specimens;

(c) Quality control in the manufacture of semiconductors.

Mass spectrometry:

In this technique, the material under examination is vaporized under a high vacuum and the vapour is bombarded a high-energy electron beam. Many of the vapour molecules undergo fragmentation and produce ions of varying size. These ions can be distinguished by accelerating them in an electric field, and then deflecting them in a magnetic field where they follow paths dictated by their mass/charge ratio (m/e) to detection and recording equipment: each kind of ion gives a peak in the mass spectrum.

Non-volatile inorganic materials can be examined by vaporizing them by subjecting them to a high-voltage electric spark.

Mass spectrometry can be used for gas analysis, for the analysis of petroleum products, and in examining semiconductors for impurities. It is also a very useful tool for establishing the structure of organic compounds.

Kinetic methods:

These methods of quantitative analysis are based upon the fact that the speed of a given chemical reaction may frequently be increased by the addition of a small amount of a catalyst, and within limits, the rate of the catalyzed reaction will be governed by the amount of catalyst present. If a calibration curve is prepared showing variation of reaction rate with amount of catalyst used, then measurement of reaction rate will make it possible to determine how much catalyst has been added in a certain instance. This provides a sensitive method for determining sub-microgram amounts of appropriate substances. The method can also be adapted to determine the amount of a substance in solution by adding a catalyst which will destroy it completely, and measuring the concomitant change in for example, the absorbance of the solution for visible or ultraviolet radiation. Such procedures are applied in clinical chemistry.

Optical methods:

Those of particular application to organic compounds are:

1. Use of a refractometer to make measurements of the refractive index of liquids. This will often provide a means of identifying a pure compound, and

can also be used (in conjunction with a calibration curve) to analyse a mixture of two liquids.

2. Measurement of the optical rotation of optically active compounds. Polarimetric measurements can likewise be used as a method of identifying pure substances, and can also be employed for quantitative purposes.

Thermal methods:

Changes in weight, or changes in energy, recorded as a function of temperature (or of time) can provide valuable analytical data. For example, the conditions can be established under which a precipitate produced in a gravimetric determination can be safely dried. Common techniques include the recording as a function of temperature or time of (a) change in weight (thermogravimetry, TG); (b) The difference in temperature between a test substance and an inert reference material (differential thermal analysis, DTA); (C) The energy necessary to establish a zero temperature difference between a test substance and a reference material (differential scanning calorimetry,DSC).

FACTORS AFFECTING THE CHOICE OF ANALYTICAL METHODS:

An indication has been given in the preceding sections of a number of techniques available to the analytical chemist. The techniques have differing degrees of sophistication, of sensitivity, of selectivity, of cost and also of time

requirements, and an important task for the analyst is the selection of the best procedure for a given determination. This will require careful consideration of the following criteria.

- The type of analysis required: elemental or molecular, routine or occasional. Problems arising from the nature of the material to be investigated. E.g. radioactive substances, corrosive substances, substances affected by water.
- Possible interference from components of the material other than those of interest.
- The concentration range which needs to be investigated.
- The accuracy required.
- The facilities available; this will refer particularly to the kinds of instrumentation which are at hand.

The time required to complete the analysis; this will be particularly relevant when the analytical results are required quickly for the control of a manufacturing process. This may mean that accuracy has to be a secondary rather than a prime consideration, or it may require the use of expensive instrumentation.

The number of analyses of similar type which have to be performed: in other words, does one have to deal with limited number of determinations or with a situation requiring frequent repetitive analyses.

Does the nature of the specimen, the kind of information sought, or the magnitude of the sample available indicate the use of non-destructive methods of

analysis as opposed to the more commonly applied destructive methods involving dissolution of the sample (possible in acid) prior to the application of normal analytical techniques.

Some information relevant to the choice of appropriate methods is given in condensed form in table, which is divided into three sections: the 'classical' techniques; a selection of instrumental methods; some 'non-destructive'methods.[2,6,7]

Table :2. Conspectus of some common quantitative analytical methods.[2]

Method	**Speed**	**Relative cost**	**Concentration range (pC)***	**Accuracy**
Gravimetry Titrimetry	SM	LL	1-21-4	HL
Coulometry Voltammetry potentiometry spectrophotometry atomic spectrometry emission(plasma) chromatography(GLC; HPLC)	S-MMM-FM-FFFFF	L-MML-ML-MM-HHM-HH	1-43-101-73-63-95-93-93-9	HMMMM MMH
Neutron activationX-ray fluorescence	SF	HH	+(a)+(b)	MH

*Pc=log101/concn, where concentration is expressed in moles per litre.

+concentration range has little significance: detection values are (a) 10^{-5}-10^{-12}g; (b) 10^{-3}-10^{-6}g.

Abbreviation: F=fast; H=High; L=Low; M=Moderate; S=Slow.

CHROMATOGRAPHY[3,8]

Chromatography (from Greek: chroma, colour and:"graphein" to write) is essentially a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases, one of which is moving fast the other that depends on differential affinities of the solute between two immiscible phases, one of which will be fix with large surface area, while the other is fluid which moves through or over the surface of the fixed phase. Tswett.M first invented the chromatographic technique in 1906.

Chromatography is a very important tool in organic chemical analysis, which involves the separation of various chemical mixtures into its individual constituents. Different types of chromatographic techniques are present for separation of compounds.

Introduction to HPLC method of analysis for drugs in combination:- [6,9-13]

Most of the drugs in multicomponent dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity,

accuracy, precision and ease of automation of this method. HPLC method eliminates tedious extraction and isolation procedures. Some of the advantages are

✓ speed (analysis can be accomplished in 20 minutes or less),

✓ greater sensitivity (various detectors can be employed),

✓ improved resolution (wide variety of stationary phases),

✓ reusable columns (expensive columns but can be used for many analysis),

✓ easy sample recovery, handling and maintenance,

✓ precise and reproducible and

✓ Calculations are done by integrator itself.

HPLC Method Development and Validation for Pharmaceutical Analysis:

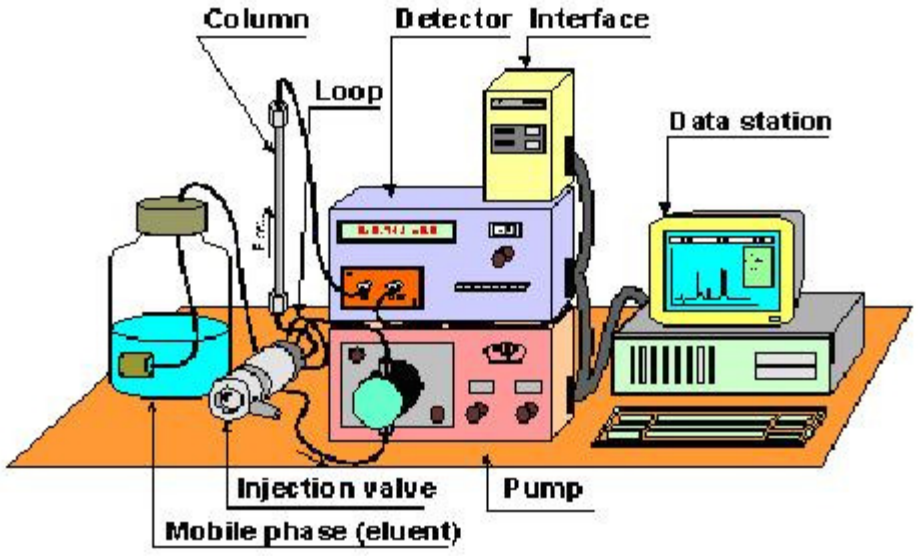


Fig.1. A schematic diagram of HPLC equipment

The wide variety of equipment, columns, eluent and operational parameters involved makes high performance liquid chromatography (HPLC) method

development seem complex. The process is influenced by the nature of the analytes and generally follows the following steps:

- Step 1 - Selection of the HPLC method and initial system
- Step 2 - Selection of initial conditions
- Step 3 - Selectivity optimization
- Step 4 - System optimization
- Step 5 - Method validation

Depending on the overall requirements and nature of the sample and analytes, some of these steps will not be necessary during HPLC analysis. For example, a satisfactory separation may be found during step 2, thus steps 3 and 4 may not be required. The extent to which method validation (step 5) is investigated will depend on the use of the end analysis; for example, a method required for quality control will require more validation than one developed for a one-off analysis.

The following must be considered when developing an HPLC method:

- Keep it simple
- Try the most common columns and stationary phases first
- Thoroughly investigate binary mobile phases before going on to ternary
- Think of the factors that are likely to be significant in achieving the desired resolution.

Mobile phase composition, for example, is the most powerful way of optimizing selectivity whereas temperature has a minor effect and would only achieve small selective changes. pH will only significantly affect the retention of weak acids and bases.

HPLC method development:-

Step 1 - Selection of the HPLC method and initial system:-

When developing an HPLC method, the first step is always to consult the literature to ascertain whether the separation has been previously performed and if so, under what conditions - this will save time doing unnecessary experimental work. When selecting an HPLC system, it must have a high probability of actually being able to analyse the sample; for example, if the sample includes polar analytes then reverse phase HPLC would offer both adequate retention and resolution, whereas normal phase HPLC would be much less feasible. Consideration must be given to the following:

Sample preparation:Does the sample require dissolution, filtration, and extraction, preconcentration or clean up? Is chemical derivatization required to assist detection sensitivity or selectivity?

Types of chromatography: Reverse phase is the choice for the majority of samples, but if acidic or basic analytes are present then reverse phase ion

suppression (for weak acids or bases) or reverse phase ion pairing (for strong acids or bases) should be used. The stationary phase should be C_{18} bonded. For low/medium polarity analytes, normal phase HPLC is a potential candidate, particularly if the separation of isomers is required. Cyano-bonded phases are easier to work with than plain silica for normal phase separations. For inorganic anion/cation analysis, ion exchange chromatography is best. Size exclusion chromatography would normally be considered for analysing high molecular weight compounds (2000).

Column dimensions: For most samples (unless they are very complex), short columns (10–15 cm) are recommended to reduce method development time. Such columns afford shorter retention and equilibration times. A flow rate of 1-1.5 mL/min should be used initially. Packing particle size should be 3 or 5 μm.

Detectors: Consideration must be given to the following:

- Do the analytes have chromophores to enable UV detection?
- Is more selective/sensitive detection required (Table I)?
- What detection limits are necessary?
- Will the sample require chemical derivatization to enhance detectability and/or improve the chromatography?

Fluorescence or electrochemical detectors should be used for trace analysis. For preparative HPLC, refractive index is preferred because it can handle high concentrations without overloading the detector.

UV wavelength: For the greatest sensitivity λ_{max} should be used, which detects all sample components that contain chromophores. UV wavelengths below 200 nm should be avoided because detector noise increases in this region. Higher wavelengths give greater selectivity.

Fluorescence wavelength:The excitation wavelength locates the excitation maximum; that is, the wavelength that gives the maximum emission intensity. The excitation is set to the maximum value then the emission is scanned to locate the emission intensity. Selection of the initial system could, therefore, be based on assessment of the nature of sample and analytes together with literature data, experience, expert system software and empirical approaches.

Step 2 - Selection of initial conditions:-

This step determines the optimum conditions to adequately retain all analytes; that is, ensures no analyte has a capacity factor of less than 0.5 (poor retention could result in peak overlapping) and no analyte has a capacity factor greater than 10–15 (excessive retention leads to long analysis time and broad peaks with poor detectability). Selection of the following is then required.

Mobile phase solvent strength:-The solvent strength is a measure of its ability to pull analytes from the column. It is generally controlled by the concentration of the solvent with the highest strength; for example, in reverse phase HPLC with aqueous mobile phases, the strong solvent would be the organic modifier; in normal phase HPLC, it would be the most polar one. The aim is to find the correct concentration of the strong solvent. With many samples, there will be a range of solvent strengths that can be used within the aforementioned capacity limits. Other factors (such as pH and the presence of ion pairing reagents) may also affect the overall retention of analytes.

Determination of initial conditions:-The recommended method involves performing two gradient runs differing only in the run time. A binary system based on either acetonitrile/water (or aqueous buffer) or methanol/water (or aqueous buffer) should be used.

Step 3 - Selectivity optimization:-

The aim of this step is to achieve adequate selectivity (peak spacing). The mobile phase and stationary phase compositions need to be taken into account. To minimize the number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined. To select these, the nature of the analytes must be considered. For this, it is useful to categorize analytes into a few basic types.

Once the analyte types are identified, the relevant optimization parameters may be selected. Note that the optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization.

Step 4 - System parameter optimization:-

This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factors or selectivity.

Step 5 - Method validation:-

Proper validation of analytical methods is important for pharmaceutical analysis when ensurance of the continuing efficacy and safety of each batch manufactured relies solely on the determination of quality. The ability to control this quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria.

Analytical method validation is now required by regulatory authorities for marketing authorizations and guidelines have been published. It is important to isolate analytical method validation from the selection and development of the

method. Method selection is the first step in establishing an analytical method and consideration must be given to what is to be measured, and with what accuracy and precision.

Method development and validation can be simultaneous, but they are two different processes, both downstream of method selection. Analytical methods used in quality control should ensure an acceptable degree of confidence that results of the analyses of raw materials, excipients, intermediates, bulk products or finished products are viable. Before a test procedure is validated, the criteria to be used must be determined.

Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B).[14,15] The US Food and Drug Administration (FDA)[16,17] and US Pharmacopoeia (USP)[18] both refer to ICH guidelines. The most widely applied validation characteristics are accuracy, precision (repeatability and intermediate precision), specificity, detection limit, quantitation limit, linearity, range, robustness and stability of analytical solutions. Method validation must have a written and approved protocol prior to use.[19]

Modes of HPLC:

In the **normal phase** mode, the stationary phase is polar and the mobile phase is nonpolar in nature. In this technique, nonpolar compounds travel faster and are eluted first. This is because of the lower affinity between the nonpolar compounds and the stationary phase and vice versa. Therefore, normal phase mode of separation is not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

Reversed phase mode is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is nonpolar hydrophobic packing with octyl or octadecyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and nonpolar compounds are retained for longer time. The different columns used are

octadecylsilane (ODS) or C18, C8, etc., (in the order of increasing polarity of the tationary phase).

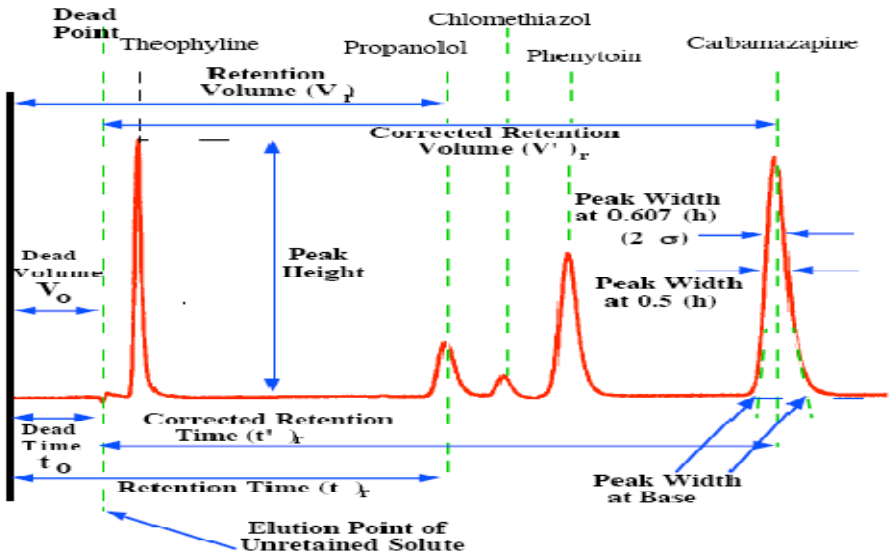


Fig.2.The nomenclature of Chromatogram

System Suitability[20,21,22]:

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility (%RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method. These parameters are measured during the analysis of a system suitability "sample" that is a mixture of main components and expected by-products. Lists of the terms to be measured and their recommended limits obtained from the analysis of the system suitability sample are given below.

Definition:

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application.

Theoretical plates per meter were calculated from the data obtained from the peak using the following expression

$$n = \frac{5.54 Vr^2}{L W_h^2}$$

Theoretical plates per column were calculated from the data obtained from the peak.

$$n = \frac{5.54 Vr^2}{W_h^2}$$

Where, 'n' is number of theoretical plates per meter, 'Vr' is the distance along the base line between the point of injection and a perpendicular dropped from the maximum of the peak of interest and 'W_h' is the width of the peak of interest at half peak height.

Table:3. System Suitability Parameters and Recommendations

System Suitability Parameters and Recommendations	
Capacity Factor (k')	The peak should be well-resolved from other peaks and the void volume, generally k'<2.0
Repeatability	RSD </= 2% for N >/= 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution (Rs)	Rs of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.
Tailing Factor (T)	T of </= 2
Theoretical Plates (N)	In general should be > 2000

If the results are adversely affected by the changes in column performance (e.g. unacceptable precision of results due to overlapping peaks), the system suitability results from these experiments will help to determine the limits for system suitability criteria.

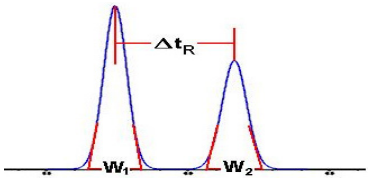
This approach facilitates the investigation of the worst case scenario, which reflects minimum performance standard used to ensure that the chromatography is not adversely affected.

The parameters that are affected by the changes in chromatographic conditions are

- Resolution (R_s),
- Capacity factor (k'),
- Selectivity (a),
- Column efficiency (N) and
- Peak asymmetry factor (A_s).

Resolution (R_s) is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture.

The resolution, R_s, of two neighbouring peaks is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 1.5. It is calculated by using the formula



$$Rs = \frac{\Delta t_R}{0.5(W_1 + W_2)}$$

Fig. 3. Resolution between two peaks

Where $\Delta t_R = t_2 - t_1$ for reliable quantitation, well-separated peaks are essential for quantitation. R_s of > 2 between the peak of interest and the closest potential interfering peak (impurity, excipient, degradation product, internal standard, etc.)

are desirable.w_1& w_2 are corresponding width at the bases of the peak of components.

There are three fundamental parameters that influence the resolution of a chromatographic separation

- capacity factor (k')
- selectivity (α)
- column efficiency (N)

These parameters provide you with different means to achieve better resolution, as well as defining different problem sources.

Capacity Factor (k') is the ratio of the reduced retention volume to the dead volume. Capacity factor, k', is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of k' ranges from 2-10. Capacity factor can be determined by using the formula,

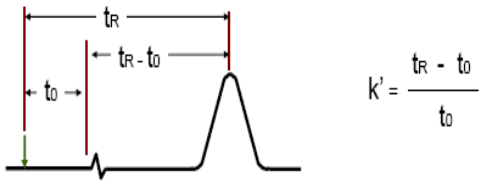


Fig. 4. Capacity factor

Where, t_R = retention volume at the apex of the peak (solute) and

t_0 = void volume of the system.

Capacity Factor (k') changes are typically due to

- Variations in mobile phase composition
- Changes in column surface chemistry (due to aging)
- Changes in operating temperature.

In most chromatography modes, capacity factor (k') changes by 10 percent for a temperature change of 5^0 C.

- **Adjusting Capacity Factor (k')** good isocratic methods usually have a capacity factor (k') in the range of 2 to 10 (typically between 2 and 5). Lower values may give inadequate resolution. Higher values are usually associated with excessively broad peaks and unacceptably long run times.

If the analytes fall outside their specified windows run the initial column test protocol to compare the results obtained with a new column.

If the shift in Capacity Factor (k') value is observed with both analytes and the column test solution, the problem is most likely due to change in the column, temperature or mobile phase composition. This is particularly true if the shift occurred gradually over a series of runs. If, however the test mixture runs as expected, the problem is most likely sample-related.

Selectivity (separation factor) (α)is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, and the ratio of its adjusted retention times. Selectivity represents the separation power of particular adsorbent to the mixture of these particular components.

This parameter is independent of the column efficiency; it only depends on the nature of the components, eluent type, and eluent composition, and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency.The ideal value of 'α' is 2. It can be calculated by using formula, $\alpha = \frac{V_2 - V_1}{V_1 - V_0} = \frac{k_1'}{k_2'}$

Where, V_0 = the void volume of the column, V_1 and V_2 =the retention volumes of the second and the first peak respectively.

Column Efficiency/ Band broadening 'N' of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5000 to 10000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16(\frac{t_R}{W_b}) \quad \text{or} \quad N = 5.54(\frac{t_R}{W_{1/2}})$$

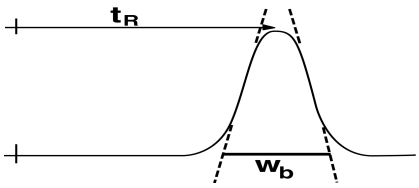


Fig. 5. Number of Theoretical Plates

Where, t_R is the retention time and W_b and $W_{1/2}$ is the peak width at base and half height respectively.

You can recognize problems in your separation due to a loss of column efficiency when the width and/or shape of all peaks are affected.

If the measured efficiency has degraded, either the column has degraded, or system band broadening has increased. At this point, check system band spreading against established benchmarks. When measuring Column Efficiency, use test conditions identical to those used in the established benchmark performance (such as test sample, flow rate, mobile phase composition and so on). Measure the column efficiency against the established performance.

Peak asymmetry factor (T_f) can be used as a criterion of column performance. The peak half width, b, of a peak at 5% of the peak height, divided by the corresponding front half width, a, gives the asymmetry factor.

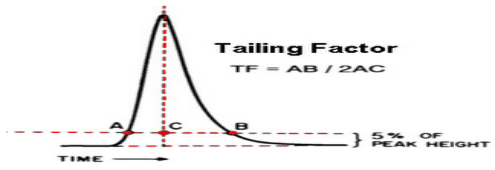


Fig. 6. Asymmetric factor

For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.Tailing factor of peak was calculated from the following expression.

Tailing factor =AB/2AC

Where, **AB** = 2*½ width of the peak at one twentieth of the peak height.

AC = ½ width of the peak at one twentieth of the peak height.

Validation Parameters:-

i) Linearity and Range

ii) Accuracy

iii) Specificity

iv) Precision

v) Limits of detection and quantitation

vi) Robustness

i) Linearity and Range: The linearity of a test procedure is its ability (within a given range) to produce results that are directly proportional to the concentration of analyte in the sample. The range is the interval between the upper and lower levels of the analyte that have been determined with precision, accuracy and linearity using the method as written. ICH guidelines specify a minimum of five concentration levels, along with certain minimum specified ranges. For assay, the minimum specified range is 80–120% of the theoretical content of active. Acceptability of linearity data is often judged by examining the correlation

coefficient and y-intercept of the linear regression line for the response versus concentration plot. The regression coefficient (r^2) is .0.998 and is generally considered as evidence of acceptable fit of the data to the regression line. The per cent relative standard deviation (RSD), intercept and slope should be calculated.

ii) Accuracy:A method is said to be accurate if it gives the correct numerical answer for the analyte. The method should be able to determine whether the material in question conforms to its specification (for example, it should be able to supply the exact amount of substance present). However, the exact amount present is unknown, which is why a test method is used to estimate the accuracy. Furthermore, it is rare that the results of several replicate tests all give the same answer, so the mean or average value is taken as the estimate of the accurate answer.

Some analysts adopt a more practical attitude to accuracy, which is expressed in terms of error. The absolute error is the difference between the observed and the expected concentrations of the analyte. Percentage accuracy can be defined in terms of the percentage difference between the expected and the observed concentrations.

Percentage accuracy tends to be lower at the lower end of the calibration curve. The term accuracy is usually applied to quantitative methods but it may also be applied to methods such as limit tests. Accuracy is usually determined by

measuring a known amount of standard material under a variety of conditions but preferably in the formulation, bulk material or intermediate product to ensure that other components do not interfere with the analytical method. For assay methods, spiked samples are prepared in triplicate at three levels across a range of 50-150% of the target concentration. The per cent recovery should then be calculated. The accuracy criterion for an assay method is that the mean recovery will be 100±2% at each concentration across the range of 80-120% of the target concentration. To document accuracy, ICH guidelines regarding methodology recommend collecting data from a minimum of nine determinations across a minimum of three concentration levels covering the specified range (for example, three concentrations, three replicates each).

iii) Specificity: Developing a separation method for HPLC involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components (placebo formulation, synthesis intermediates, excipients, degradation products and process impurities) is compared with the response of a solution containing only the analyte. Other potential sample components are generated by exposing the analyte to stress conditions sufficient to degrade it to 80–90% purity. For bulk pharmaceuticals, stress conditions such as heat (50–60 ºC), light (600 FC of UV), acid (0.1 M HCl), base (0.1 M NaOH) and oxidant (3% H_2O_2) are typical.

For formulated products, heat, light and humidity (70-80% RH) are often used. The resulting mixtures are then analysed, and the analyte peak is evaluated for peak purity and resolution from the nearest eluting peak.

Once acceptable resolution is obtained for the analyte and potential sample components, the chromatographic parameters, such as column type, mobile phase composition, flow rate and detection mode, are considered set. An example of specificity criterion for an assay method is that the analyte peak will have baseline chromatographic resolution of at least 2.0 from all other sample components. In this study, a weight of sample placebo equivalent to the amount present in a sample solution preparation was injected to demonstrate the absence of interference.

iv) Precision: Precision means that all measurements of an analyte should be very close together. All quantitative results should be of high precision - there should be no more than a ±2% variation in the assay system. A useful criterion is the relative standard deviation (RSD) or coefficient of variation (CV), which is an indication of the imprecision of the system

According to the ICH[14],precision should be performed at two different levels - repeatability and intermediate precision. Repeatability is an indication of how easy it is for an operator in a laboratory to obtain the same result for the same batch of material using the same method at different times using the same

equipment and reagents. It should be determined from a minimum of nine determinations covering the specified range of the procedure (for example, three levels, three repetitions each) or from a minimum of six determinations at 100% of the test or target concentration.

Intermediate precision results from variations such as different days, analysts and equipment. In determining intermediate precision, experimental design should be employed so that the effects (if any) of the individual variables can be monitored. Precision criteria for an assay method are that the instrument precision and the intra-assay precision (RSD) will be ≤2%.

v)Limits of detection and quantitation:The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. It is expressed as a concentration at a specified signal: noise ratio,[14] usually 3:1. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The ICH has recommended a signal: noise ratio 10:1. LOD and LOQ may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve(s) at levels approximating the LOD according to the formulae:

LOD=3.3(SD/S) and LOQ=10(SD/S).

The standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines. The method used to determine LOD and LOQ should be documented and supported, and an appropriate number of samples should be analysed at the limit to validate the level.

vi) Robustness: Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters. It also provides some indication of the reliability of an analytical method during normal usage. Parameters that should be investigated are percent organic content in the mobile phase; pH of the mobile phase; buffer concentration; temperature; and injection volume. These parameters may be evaluated one factor at a time or simultaneously as part of a factorial experiment. The chromatography obtained for a sample containing representative impurities when using modified parameter(s) should be compared with the chromatography obtained using the target parameters.[20,21,22]

Finally the conclusion is, Method development involves a series of sample steps; based on what is known about the sample, a column and detector are chosen; the sample is dissolved, extracted, purified and filtered as required; an eluent survey (isocratic or gradient) is run; the type of final separation (isocratic or gradient) is determined from the survey; preliminary conditions are determined for the final separation; retention efficiency and selectivity are optimized as required

for the purpose of the separation (quantitative, qualitative or preparation); the method is validated using ICH guidelines. The validated method and data can then be documented.

REVIEW OF LITERATURE

- J. V.Shanmukha Kumar et al., have reported validation of analytical procedures for determination of Mebendazole.
- Acharjya sasmita kumari et al., have reported UV-Spectroscopic methodsfor estimation of Mebendazole in pharmaceutical preparations[26].
- Khedkar amol et al., have reported spectrophotometric method for analysis of Mebendazole.
- Prithvuraj S Yadav et al., have reported determinatio nof Mebendazole in bulk and its tablet dosage forms by UV spectroscopic method[28].
- Ramzia.I.El-Bagary et al., have reported fluorimetric and colorimeric methods for the determination of some anti migraine drugs[29].
- Madhukar. A et al., have reported analytical method development and validation of Mebendazole Chewable tablets by RP-LC[30].
- Devprakash et al., have reported estimation of Mebendazole by RP-HPLC method in bulk and dosage form[31].
- Gopichand.I et al., have reported HPLC method for quantitative determination of Mebendazole, an anti migraine agent in pharmaceutical dosage forms and purity evaluation in bulk drugs[32].
- Punna venkateshwarlu et al., have reported a validated and simplified RP-HPLC of Mebendazole from bulk drugs[33].

- Murthy T.E.G.k. et al., have reported reverse phase HPLC method developmment for the estimation of Mebendazole from formulated oral-dispersable tablets[34].
- Sachin S Jagathap et al., have reported stability indicating reverse-phase high-performance liquid chromatographic method for the determination of Mebendazole in bulk and its pharmaceutical formulations[35].
- El-Bagary RI et al., have reported two chromatographic methods for the determination of some anti migraine drugs[36].
- Devprakash dahiya et al., have reported determination of Mebendazole in bulk and its tablet dosage forms by HPTLC method[37].
- Syama Sundar B et al., have reported development and validation of HPTLC method for the estimation of Mebendazole benzoate in bulk and tablets[38].
- Trinath. M et al., have reported development and validation of spectrophotometric method for simultaneous estimation of Albendazole and Naproxen sodium in tablet dosage form[39].
- Buridi. Kalyana Ramu et al., have reported estimation of Albendazole in bulk and formulations by visible spectrophotometry using aromatic aldehydes[40].
- C.R.Shah et al., have reported developmant and validation of a HPTLC method for the estimation of Albendazole in tablet dosage forms[41].
- P. Vivek sagar et al., have reported simultaneous estimation of Mebendazoleand Albendazoleby RP-HPLC method in bulk forms[42].

- Suneetha et al., have reported a validated RP-HPLC method for estimation of Albendazole in pharmaceutical dosage forms[43].
- Tariq Mahmood Ansari et al., have reported a novel spectrophotometric method for determination of Albendazole in pharmaceutical formulations[44].
- Madhusudhanareddy Induri et al., have reported a validated RP-HPLC method for the quantification of Albendazole in tablet dosage form[45].
- Ramakotaiah mogili et al., have reported determination of Mebendazole in human plasma by liquid chromatography stable isotope dilution electrospray MS-MS for application in bioequivalence study[46].
- Vishwanathan. k et al., have reported determination of antimigraine compounds Mebendazole andAlbendazole in human serum by liquid chromatography/electrospray tandem mass spectrometry[47].
- Guo. JF et al., have reported determination of Mebendazole in human plasma by liquid chromatographic electrospray tandem mass spectrometry : application to a pharmacokinetic study[48].
- Qin yong ping et al., have reported determination of Mebendazole in human plasma by RP-HPLC with fluorescence detection[49].
- Chen. J et al., have reported liquid chromatographic method for the determination of Mebendazole in human plasma[50].

AIM AND OBJECTIVES

The durg analysis plays an important role in the development, manufacture and therapeutic use of drugs. Most of the pharmaceutical industries do the quantitative chemical analysis to ensure that the raw materials used and the final product thus obtained meet certain specification and to determine how much of each components are present in the final products. Standard analysis analysis procedure for newer drugs or formulation may not be available in pharmacopoeias; hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid.

Aim:

To develop and validate new RP-HPLC method for the Assay and chromatographic purity of Mebendazole Chewable tablets 500mg.

Objective:

Literature survey reveals that only few analytical methods have been reported for the estimation of Mebendazole in pharmaceutical dosage form.

Hence an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for the estimation of Mebendazolein pharmaceutical dosage forms using High Performance Liquid Chromatography techniques. This method can also be applied for estimation of pure drug.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

To develop and validate Reversed phase high performance liquid chromatography method for estimation of Mebendazolein pharmaceuticaldosage forms.

An attempt has been made to develop and validate all the above mentioned methods to ensure their accuracy, precision, and other analytical method validation parameters as mentioned in the ICHGuidelines Q2 (R1).

In summary, the primary objective of proposed work is to:

- Since there are only few methods are available for the determination of Mebendazole. The present work is an attempt to estimate the same by different method such as New HPLC method.
- Develop new, simple, sensitive, accurate, and economical analytical method for the Estimation of Mebendazoleby HPLC.
- Validate the proposed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.
- To compare the results of the various methods.

PLAN OF WORK

- Gather / generate background information obtain physic-chemical properties.
- Determine if special handling / treatment of sample is need.
- From physicochemical properties select detector parameters.
- Calculate approximately separation parameters/isocratic or gradient mode.
- Perform forced degradation experiments to challenge method.
- Optimization separation conditions.
- Summarize methodology and finalize documentation.
- Analysis of marketed formulation and validate method.

DRUG PROFILE

MEBENDAZOLE

Mebendazole is a Methyl 5-benzoyl-2-benzimidazolecarbamate drug used for the treatment of Anthelmintic (Nematodes). It is a selective 5-benzoyl-1*H*-benzimidazol-2-yl receptor type agonist.

Structure:

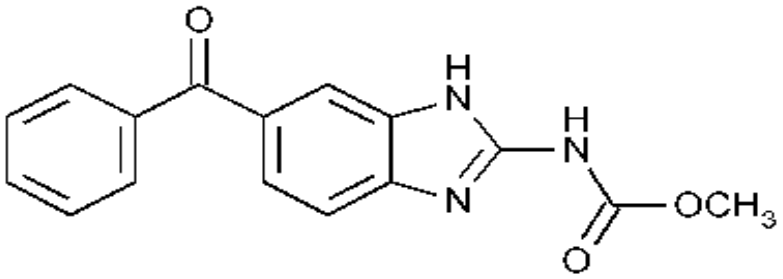


Fig.7. Structure of Mebendazole

IUPAC NAME:

Carbamic acid,(5-benzoyl-1*H*-benzimidazol-2-yl),methyl ester.
Methyl 5-benzoyl-2-benzimidazolecarbamate [*31431-39-7*].

PHYSICAL PROPERTIES:

i) Chemical Formula: $C_{16}H_{13}N_3O_3$

ii) Molecular weight: 295.29

iv) Melting point: 288.5°C.

vi) PKa: 6.6 (Double bonded nitrogen)

i) Storage temperature: 0-6°C

MEBENDAZOLE CHEMICAL PROPERTIES,USAGE,PRODUCTION

Chemical Properties

White Amorphous Powder

Usage

Anthelmintic (Nematodes)

General Description

White to slightly yellow powder. Pleasant taste. Practically water insoluble.

Air & Water Reactions

Insoluble in water.

Reactivity Profile

Mebendazole is a carbamate ester-amine. Amines behave as chemical bases. Carbamates are chemically similar to, but more reactive than amides. Like amides they form polymers such as polyurethane resins. Carbamates are incompatible with strong acids and bases, and especially incompatible with strong reducing agents such as hydrides. Flammable gaseous hydrogen is

produced by the combination of active metals or nitrides with carbamates. Strongly oxidizing acids, peroxides, and hydroperoxides are incompatible with carbamates.

Fire Hazard

Flash point data for Mebendazole are not available; however, Mebendazole is probably combustible

Packaging and storage: Preserve in well-closed containers.

USP Reference standards á11ñ**—***USP Mebendazole RS*.

Identification, *Infrared Absorption* á197Kñ.

Loss on drying á731ñ**:** Dry it at 105°for 4hours:it loses not more than 0.5%of its weight.

Residue on ignition á281ñ: not more than 0.1%.

Heavy metals, *Method II*á231ñ: 0.002%.

Mechanism of action[24]:

Two hypotheses are described for Anthelmintic drug.

1) Anthelmintic cause constriction of intracranial blood vessels and dilation of carotid arteriovenous anastomoses in head.
2) Anthelmintic may block the release of prooinflammatory neuropeptides at the level of the nerve terminal in the perivascular space.

Pharmacokinetics:

- oral bioavailability is 45%
- peak plasma levels within 1-1.5hrs.
- metabolism via oxidative deamination by MAO-A.

Adverse effects:

- rare but serious cardiac events have been associated with 5HT1 agonists

  -coronary artery vasospasm

  -transient myocardial ischemia

  -myocardial infarction

  -asthenia and fatigue

  -tightness/pain in chest, neck, jaw

  -drowsiness and dizziness

Contraindications:

- Contraindicated in patients with cardiovascular, hepatic and renal diseases.

MATERIALS AND METHODS

Estimation of Mebendazole by RP-HPLC method.

Apparatus and software

The Waters Alliance 2695, equipped with a UV detector column oven heater / cooler and auto-sampler.

Compact LC HPLC system consisting of gradient pump(4MPa or 40barr), rheodyne injector, UV variable detector and waters syringe (25µl) was used. The separations were achieved on a Zorbax SB C18 (150x 4.6)mm, id 5.0µm particle size column with UV detection at 250nm. Analytical weighing balance (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON-230VAC, 50Hz), and vacuum pump (SUPER FIT 110336), Millipore filtration kit (TARSONS) with GHP Acrodisc® 25mm syringe filters with 0.45µm GHP membrane (available from Pall Life Sciences, Part Number-4506T) for solvents and sample filtration were used throughout the experiment. The Waters Empower software was used for acquisition, evaluation and storage of chromatographic data.

Reagents and Pharmaceutical Preparations

Analytically pure sample of Mebendazole procured as gift sample byJanssen and Janssenla boratories (Mumbai). The drug is used without further purification. HPLC grade Methanol (Merck), Pharmaceutical formulation Mebendazole Chewable tablets R017635 G002(label claim 500mg) batch no.Smarathe-02-027 and Smarathe-025/03, Mfg. Lic. No. 164/MN/AP/95/F/R

Manufactured by Janssen Pharma Limited, was used in the HPLC. HPLC grade water obtained in-house by using Direct-Q®3 with pump (Elec. Ratings: 100-230V of 50-60Hz 100VA) water purification system (made in France) and GR grade orthophosphoric acid 88% were used in HPLC study.

Selection of Mobilephase

Anumberof trials were made to findout the ideal solvent system (mobilephase) for eluting the drug.Themobilephasegradient programming containing Water: Acetonitrile (80:20), 0.1% Trifluoro acetic acid buffer: Acetonitrile (80:20), Water: Acetonitrile (90:10),0.025% Trifluoro acetic acid buffer: Acetonitrile (80:20) and 0.025% Trifluoro acetic acid buffer: Acetonitrile (90:10) was tried. Better peak and adequate retentiontime were obtained with the ratio of 0.025% Trifluoro acetic acid buffer: Acetonitrile (90:10).

Mobile Phase A: 0.025% Trifluoro Acetic Acid

Transfer using a pipette, 0.25-mL Trifluroacetic acid to 1000mL volumetric flask containing about 500mL of Milli-Q or HPLC grade water and mix well.

Dilute to volume with Milli-Q or HPLC grade and degas online.

Mobile Phase A may be used for up to 7 days.

Mobile Phase B: Acetonitrile

Preparation of Dilution Solvent

Prepare a mixture of water and methanol v/v 40/60 in a suitable volumetric flask.

Allow the dilution solvent to equilibrate to room temperature before use.

Preparation of Reference Solutions

Standard Solution 1

A. Weigh accurately 50.0 mg (± 2.0 mg) of Mebendazole (= R017635) reference material (= q_r in mg) into a 50-mL amber glass volumetric flask.

B. Add 15-mL formic acid and sonicate to dissolve.

C. Add approximately 30-mL dilution solvent and mix well.

D. Equilibrate to room temperature.

E. Dilute to volume with dilution solvent and mix well.

Second Standard Solution 2

Repeat procedure for preparation of Standard Solution Mebendazole.

Reference Solution 1 (at 100% level)

A. Transfer using volumetric pipette 5-mL of standard solution Mebendazole to 50-mL amber glass volumetric flask.

B. Dilute to volume with dilution solvent and mix well.

C. Just before the appropriate filling of the auto-sampler vial, shake up the volumetric flask manually vigorously

Reference Solution 2 (at 100% level)

A. Transfer using volumetric pipette 5-mL of second standard solution Mebendazole to 50-mL amber glass volumetric flask.

B. Dilute to volume with dilution solvent and mix well.

C. Just before the appropriate filling of the auto-sampler vial, shake up the volumetric flask manually vigorously

Sample Solution

A. Accurately weigh 10 no of Mebendazole 500mg Chewable tablets and determine the mean weight. Grind the tablets as homogeneous powder.

B. Weigh and transfer equivalent to 100mg of mebendazole powder (about 200mg) into a 100mL amber glass volumetric flask.

C. Add 30-mL formic acid by graduated cylinder.

D. Sonicate for 20minutes.

E. Add 60-mL of dilution solvent and mix well.

F. Equilibrate to room temperature and dilute to volume with dilution solvent and shake up manually vigorously. Keep aside for 10minutes to sediment the particle.

G. Transfer, using a volumetric pipette 10-mL of this solution to 100-mL amber glass volumetric flask and dilute to volume with dilution solvent and mix well.

H. Filter the Sample Solution through 0.45 µm GHP Acrodisc membrane filter (Part no.-4560T).

I. Discard the first 3 mL filtrate into a waste container, not back into the volumetric flask.

J. Fill the auto-sampler vial to the appropriate height with filtrate.

Blank Solution

A. Transfer 30-mL Formic Acid to 100-mL amber glass volumetric flask.

B. Sonicate for 20min.

C. Add approximately 60-mL of dilution solvent and mix well.

K. Equilibrate to room temperature and dilute to volume with dilution solvent and shake up manually vigorously. Keep aside for 10minutes to sediment the particle.

D. Transfer, using a volumetric pipette 10-mL of this solution to 100-mL amber glass volumetric flask and dilute to volume with dilution solvent and mix well.

E. Filter through 0.45 µm GHP Acrodisc membrane filter (Part no.-4560T).

F. Discard the first 3 mL filtrate into a waste container, not back into the volumetric flask.

G. Fill the auto-sampler vial to the appropriate height with filtrate

PROCEDURE

Remarks[i]

Chromatographic Parameters Settings:

Agilent system:	Peak width: > 0.2min (4s)
	Slit (nm): 1
Alliance®system:	sampling rate: 2.0
	Method channel bandwidth: 1.2

HPLC Conditions for Identification and Content uniformity

Column:	Zorbax SB C18 (150x4.6)mm ID, 5.0µm particle size or equivalent (*A column is equivalent if it meets the system suitability criteria)
Column Temperature:	40°C± 2.0°C
Auto-Sampler Temperature:	5°C
Flow Rate:	1.50 mL/min
Injection volume:	10 µL
Detection:	UV at 250 nm
Run time:	35 min
Elution mode:	Gradient elution

A linear gradient is programmed as described in the following Table 4.

Table 4: Linear gradient is programme

Time(min.)	0	20	29	30	35
% A	90	70	30	90	90
% B	10	30	70	10	10

Equilibrate HPLC and condition the column with the mobile phase gradient until a stable gradient profile is obtained. The system suitability solutions, blank, reference solutions and samples are injected according to the current active procedure of the executing lab.

Remarks[i]

Use needle wash solvent to avoid carryover

Needle Wash: Mixture of Methanol and Formic acid in the ratio 70:30 v/v.

Waters HPLC: Extended needle wash mode

Agilent HPLC: Wash vial filled with needle wash solvent

Sequence

It is recommended to purge the injector by injecting at least 6 times 100 µL Dilution Solvent.

The following injections, at a minimum, should be run:

Blank injection: inject blank (additional Blanks may be run until an acceptable baseline is obtained).

A. Single injection of Reference Solution 2.

B. Five successive injections of Reference Solution 1.

C. Injection(s) of Sample Solution(s) (1 injection per Sample preparation).

D. Single injection of Reference Solution 1 after 12 or less Sample Solution injections and after the last Sample Solution injection (= Control Reference).

System Suitability

A. The percent relative standard deviation (% RSD) for the area for Mebendazole of five replicate injections of the Reference Solution 1 should be less than or equal to 2.0 %.

B. The percentage recovery of Mebendazole in Reference Solution 2 is 98.0% ≤ % Recovery ≤ 102.0%.

C. The percentage recovery of Mebendazole in Reference Solution 3 at the reporting threshold value is 70.0% ≤ % Recovery ≤ 130.0%.

D. The tailing factor (T) for the Mebendazole peak from first replicate of reference solution must be less than 2.0, as calculated by the current USP method

E. The Resolution factor (R) between (R019020 and R017635) in the selectivity solution must be greater than 2.5 as calculated by current USP method.

F. The reference solution must be injected as a calibration check after each series of maximum 12 sample injections and after the last sample injection. The percentage Recovery of Mebendazole is 98.0% ≤ % Recovery ≤ 102.0%.

CHROMATOGRAPHIC CHARACTERISTICS

The identity of the degradation products are assigned based on a comparision of relative retention times (RRT) with the experimentally determined retention times listed in the table below. Degradation products are qualified based on the Relative Response Factor (RRF). For unspecified and unidentified impurities, not listed in the table, the RRF is assumed to be 1.00.

Model Compounds	RRT	RT	RRF	LOD	LOQ
R018986 (*)	0.70	0.10%	TBD	TBD	TBD
R019020 (**)	0.90	NA	NA	NA	NA
R017635 (Active)	1.00	0.10%	TBD	TBD	TBD

(*) degradation product

(**) system suitability compound or (**) for system suitability only

TBD: to be determined

RT: reporting threshold

LOD: limit of detection

LOD: limit of Quantitation

NA: Not applicable

Guide retention time of mebendazole: Approx. 17.0 minutes

List of detectable unspecified products

Model Compounds	RRT
R039801 (***)	0.76
R030226 (***)	0.94
R039553 (***)	1.10
R018194 (***)	1.19
R017857 (***)	1.23
R037725 (***)	1.47

(***) synthesis impurities

RT/IT/QT according to ICH

	Percentage
RT (=Reporting threshold)	0.10%
IT (=Identification threshold)	0.20%
QT (=Qualification threshold)	0.20%

Chemical Names of impurities and Active

R018986	(2-amino-1H-benzimidazol-5-yl)phenylmethanone
R039801	(2-amino-1-methyl-1H-benzimidazol-5-yl)phenylmethanone
R019020	(2-hydroxy-1H-benzimidazol-5-yl)phenylmethanone
R017635	Methyl(5-benzoyl-1H-benzimidazol-2-yl)cabamate
R039553	Methyl(5-benzoyl-1-methyl-1H-benzimidazol-2-yl)cabamate
R018194	Ethyl(5- benzoyl-1H- benzimidazol-2-yl)cabamate
R017857	Methyl[5-(4-methylbenzoyl)-1H-benzimidazol-2-yl)cabamate
R037725	N,N-bis(5-benzoyl-1H- benzimidazol-2-yl)urea
R030226	N (5-benzoyl-1H- benzimidazol-2-yl) acetamide

CALCULATION

The described formulas are used for manual calculation of the results. A validated chromatographic data system can also be applied.

For multiple injections the mean of all individual results must be calculated.

Identification of Mebendazole

The retention time of Mebendazole in the sample solution is similar to the retention time of Mebendazole in the reference solution.

Second Identification of Mebendazole

The comparision of the maximum wavelength of the Mebendazole from

the reference solution and the sample solution must be between ± 2nm.

Guide wavelength of the Mebendazole: 235nm

Recovery of Mebendazole

Calculate the percentage recovery of Mebendazole in the reference solutions (control reference solutions and second reference solution), by the formula:

$$\% = D_r \times \frac{r_{rx}}{r_r} \times \frac{q_x}{q_{rx}} \times 100$$

Where,

D_r Dilution factor = 1 for calculation of Mebendazole in the 'second reference solution' and the 'control reference solution '

Dilution factor = 1000 for calculation of Mebendazole in the reporting threshold solution'

q_r weight (in mg) of the Mebendazole reference material in the 'reference solution'

q_{rx} weight (in mg) of Mebendazole in the 'second reference solution' or 'reference solution' for calculation of control references and reporting threshold level

r_{rx} peak area of Mebendazole in the 'second reference solution' or 'control reference solution'

r_r mean peak area of Mebendazole in the 'reference solution'

Assay of Mebendazole

Measure the peak area of Mebendazole in each chromatogram.

Calculate the quantity (Q in mg) of Mebendazole in the portion of Mebendazole chewable tablets 500mg against the stated amount, by the formula:

$$Q = Sr \times qr \times \frac{r_m}{r_r} \times \frac{\Pr}{F'_r}$$

Where,

S_r *2.0* = scaling factor = ratio of volume of sample solution to the volume of reference solution (Mebendazole) taking into account the dilution.

q_r weight (in mg) of the Mebendazole reference material in the 'reference solution'

r_m Peak area of Mebendazole in sample solutions

r_r Mean peak area of Mebendazole in the 'reference solution'

P_r Purity factor of Mebendazole

F'_r 1 = salt/base factor of Mebendazole

Calculate the percentage of Mebendazole in the Mebendazole 500mg chewable tablets by the formula:

$$\% = \frac{Q}{Qth} \times \frac{G}{qs} \times 100$$

Where,

G Mean weight (in mg) of one Mebendazole 500mg chewable tablets.

Q_{th} 500 = Theretical conc. of R017635 (in mg/tablet) in Mebendazole 500mg chewable tablets.

q_s Weight (in mg) of Mebendazole 500mg chewable tablet

Powder in sample solution.

Assay of Degradation Products

Calculate the quantity (Q_i in mg) of the degradation products and unidentified peaks expressed as Mebendazole in the portion of Mebendazole chewable tablets 500mg taken, by the formula:

$$Qi = Si \times qr \times \frac{r_{im}}{r_r} \times \frac{\Pr}{F'_r}$$

Where,

S_i *2.0* = scaling factor = ratio of volume of sample to the volume of reference solution (Mebendazole) taking into account the dilution.

q_r weight (in mg) of the Mebendazole reference material in the 'reference solution'

$r_{i,m}$ Peak area of impurity in sample solutions

r_r Mean peak area of Mebendazole in the 'reference solution'

P_r Purity factor of Mebendazole

F'_r 1 = salt/base factor of Mebendazole

Calculate the percentage of the impurity in the Mebendazole 500mg chewable tablets by the formula:

$$\%impurity = \frac{Qi}{Qth} \times \frac{G}{qs} \times \frac{1}{RRFi} \times 100$$

Where,

G Mean weight (in mg) of one Mebendazole 500mg chewable tablets.

Q_{th} 500 = Theretical conc. of R017635 (in mg/tablet) in Mebendazole 500mg chewable tablets.

q_s Weight (in mg) of Mebendazole 500mg chewable tablet

Powder in sample solution.

RRF_i Relative response factor of the impurity (expressed as the anhydrous parent compound) with regard to mebendazole, for unspecified and or unidentified impurities $RRF_i = 1$

VALIDATION OF ANALYTICAL METHOD

This validation report summarizes the results of the method validation and demonstrates that the test method is suitable for identification and assay of R017635 and determination of its impurities in:

R017635 G002, Mebendazole Chewable 500mg Tablets.

The validation characteristics are tested in accordance to the method validation protocol DS-VAL-66196, Version: 2.0

Some numbers are reported with less decimals than those used for the calculations. Therefore small differences are possible when results are calculated with the numbers as mentioned in this report.

The compounds that are evaluated during this validation are listed in Table 5.

Table 5: Table of Compounds

Compound	Batch	P	F'	Description
R017635	ZR017635PUA721	0.997	1.000	Reference material
R018986	FBEN0057_085_3	0.999	1.000	Validated compound
R019020	COCS_0074_052_1	0.990	1.000	Selected compound
R030226	COCS0111_087_1	0.996	1.000	Synthesis impurity
R039553	PROF0001_052_1	0.961	1.000	Synthesis impurity
R017857	FBEN0056_049_2	0.990	1.000	Synthesis impurity
R018194	MBER_0005_068_4	0.957	1.000	Synthesis impurity
R037725	FBEN0056_079_4	0.984	1.000	Synthesis impurity
R039801	WVLA_0042_099_2	0.994	1.000	Synthesis impurity
R017635 G002	Smarathe-02-027	NA	NA	Sample
R017635 G002	Smarathe-02-025/03	NA	NA	Sample
Placebo	Smarathe-02-036	NA	NA	Placebo without Active

P: purity factor

F': salt factor

Specificity and Identification

Specificity: Test Results and Acceptance Criteria:

Specificity is shown by analyzing the following solutions: blank solution, placebo, stressed placebo: Batch Smarathe-02-036 stored at 70°C for 5days, stressed placebo spiked with 0.50%, w/w all revelant impurities, selectivity solution/SST2, reference solution, 0.50%, w/w of each impurity, mixture of each compound in a concentration level of 0.50%, w/w next to 100%, w/w of Mebendazole and placebo at nominal concentration, sample solution (R017635 G002): batch Smarathe-02-027 and stressed samples (R017635 G002): batch Smarathe-02-027 stored at 70°C for 5 days.

Peak purity of the Mebendazole is checked by HPLC-PDA. The reference solution, the sample solution and the stressed sample solutions must be examined.

Identification of the Mebendazole is based on retention time will be evaluated over the entire method validation.

Second identification of the Mebendazole with HPLC-PDA will also be demonstrated. A reference solution, a sample solution will be analysed.

Acceptance Criteria:

To be reported:

The relative retention time (RRT) of each compound is reported and specificity is shown by means of an overlay of the chromatograms.

Criteria:

Table 6: Specificity: Acceptance criteria

Parameter	Criteria
Blank peaks	Preferably absent
Placebo peaks	Should be separated from Mebendazole and from the specified/validated impurities

Validated impurities	Should be separated from Mebendazole and from each other
Selected impurities	Should be separated from Mebendazole and from the validated impurities.

Parameter	Criteria
Peak purity of API using PDA	The UV spectra of Mebendazole at front, middle and tail are comparable or peak purity is calculated with software (Empower) and should confirm that the peak of the Mebendazole is pure. The Purity Angle (PA) must be smaller than the Purity Threshold (PT)

Table 7: Identification: Acceptance criteria

Parameter	Criteria
Identification of the Mebendazole based on retention time	Matching retention time windows (±5% of retention time of reference)
Identification of the Mebendazole with HPLC-PDA	The maximum of the spectrum of the Mebendazole in the sample may not differ more than 2 nm from the maximum of the spectrum of the Mebendazole in the reference. The profiles of the spectra of sample and reference must correspond. No interference of placebo.

Test Results for specificity:

Elution order has been determined for the impurities

Table 8: Relative Retention Times

Compound	Relative Retention Time
R018986: Validated compound	0.70
R019020: Selected compound	0.92
R039801: Synthesis impurity	0.76
R030226: Synthesis impurity	0.94
R039553: Synthesis impurity	1.09
R018194: Synthesis impurity	1.19
R017857: Synthesis impurity	1.23
R037725: Synthesis impurity	1.46
R017635: Mebendazole (API)	1.00

- ✓ No blank peak interferes with peaks of interest.
- ✓ All placebo peaks are separated from Mebendazole and from the specified/validated impurity.
- ✓ All validated impurity are separated from Mebendazole and from each other.
- ✓ The selected impurities are separated from Mebendazole and from the validated impurity.

The peak purity confirms (result from Empower software) that the peak of the Mebendazole is pure because the purity angle (PA) is smaller than the purity threshold (PT).

Table 9: Specificity: Peak Purity of Mebendazole by PDA

Sample	Peak Purity
Reference solution	Pass
Sample solution (R017635 G002): batch Smarathe-02-027	Pass
Stressed sample (R017635 G002): batch Smarathe-02-027, stored at 70°C for 5 days.	Pass

Fig 8: Specificity: Overlay Chromatograms of the mixture, the selectivity solution and the separately injected impurities.

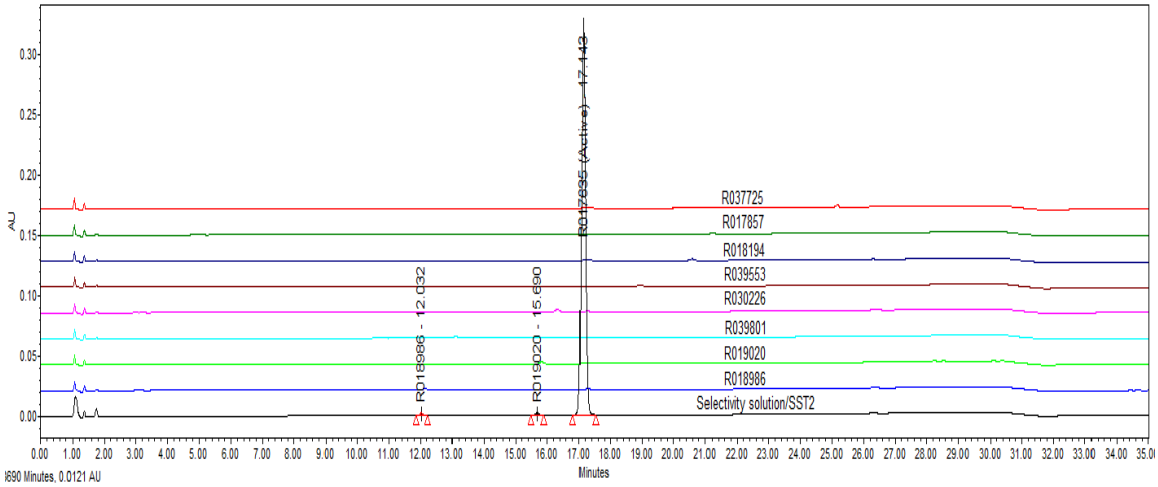


Fig 9: Specificity: Overlay Chromatograms of blank, placebo, reference solution at reporting threshold, reference solution at 100% and Mixture of each compound in a concentration level of 0.50 %, w/w next to 100.0 %, w/w of Mebendazole and placebo at nominal concentration.

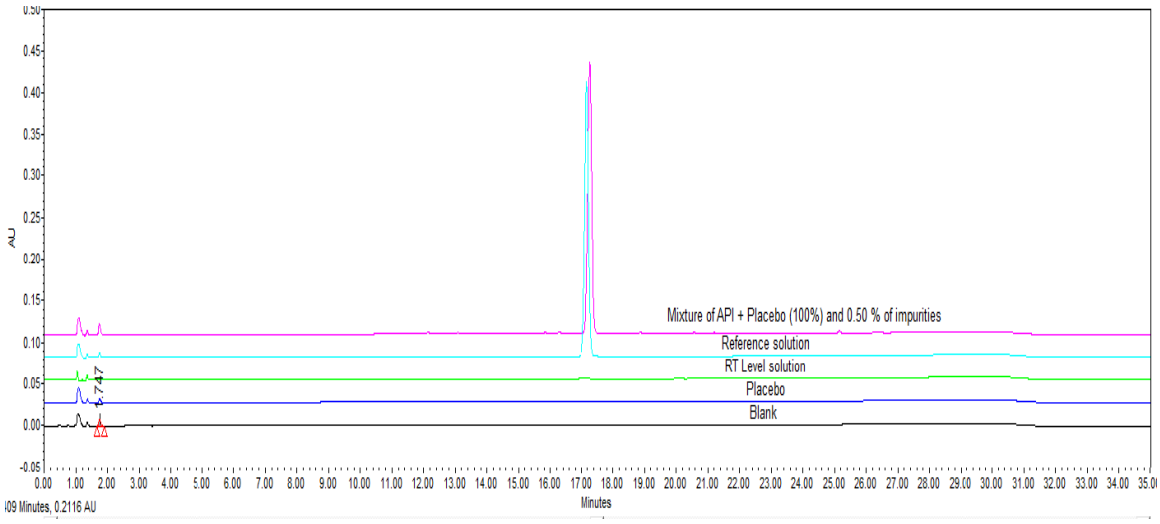
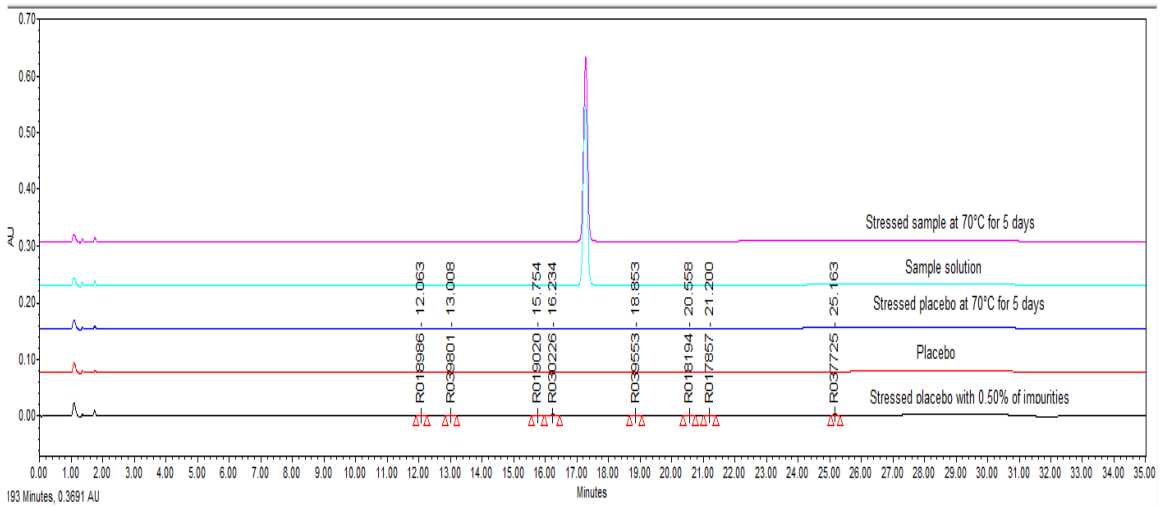


Fig 10: Specificity: Overlay Chromatograms of placebo, stressed placebo (batch Smarathe-02-036 stored at 70 °C for 5 days), stressed placebo spiked with 0.5%w/w all relevant impurities, sample solution (R017635 G002): batch Smarathe-02-027, stressed sample (R017635 G002): batch Smarathe-02-027 stored at 70°C for 5 days.



Test results for Identification of the Mebendazole based on retention time:

The retention time windows were evaluated over the entire method validation. The retention time range of the Mebendazole in the sample solution is ± 5% of the retention time of the Mebendazole in the first injection of the reference solution.

Test results for Identification of the Mebendazole with HPLC-PDA:

Table 10: Identification of Mebendazole with HPLC-PDA

Solution	Maximum	Criteria
Placebo	-	
Reference solution	235.3 nm	
Sample (R017635 G002) batch smarathe-02-027	235.3 nm	
Difference between the maximum wavelength of the spectrum of the Mebendazole in the sample solution and the maximum wavelength of the spectrum of the Mebendazole in the reference solution	0 nm	≤ 2nm

- ✓ The profile of the spectra of spiked placebo/sample and reference corresponds.
- ✓ No interference of placebo.

Conclusion:

The results prove that the method is specific and can be used for identification of the Mebendazole.

System Repeatability (Precision): Mebendazole

System Repeatability: Test Results and Acceptance Criteria:

The system repeatability is calculated as % RSD of 5 injections of the same reference solution.

Table 11: System Repeatability

Determination	Area Counts µV.sec
1	2911272
2	2937799
3	2921722
4	2895892
5	2898414
Mean:	2913020

RSD (%)	Criterion (%)
0.6	≤ 1.0

Accuracy and Analysis Repeatability (Precision): Mebendazole

Accuracy and Precision: Active (Mebendazole)

Accuracy at high concentration levels: Test Results and Acceptance Criteria:

The accuracy is assessed using 9 determinations covering the specified range at 3 concentration levels: 80.0, 100.0 and 120.0%, w/w. Each concentration is prepared 3 times by separate weighing and contains placebo at 100% level. The accuracy is calculated as the % recovery and the mean recovery is calculated at each concentration level.

Table 12: Accuracy: At high Concentration Levels

Concentration Theoretical (%)	Concentration Practical (mg/mL)	Area Counts µV.sec	Recovery (%)	Criteria (%)
80.0 % (1)	0.08031	2347049	100.2	97.0-103.0
80.0 % (2)	0.08095	2365725	101.0	97.0-103.0
80.0 % (3)	0.08140	2379077	101.7	97.0-103.0
Mean recovery:			101.0	98.0-102.0
100.0 % (1)	0.1017	2972730	101.6	97.0-103.0
100.0 % (2)	0.1014	2963285	101.3	97.0-103.0
100.0 % (3)	0.1014	2963687	101.4	97.0-103.0
Mean recovery:			101.4	98.0-102.0
120.0 % (1)	0.1196	3497668	99.6	97.0-103.0
120.0 % (2)	0.1215	3551706	101.2	97.0-103.0
120.0 % (3)	0.1213	3545434	101.0	97.0-103.0
Mean recovery:			100.6	98.0-102.0

Analysis Repeatability at high concentration levels: Test Results and Acceptance Criteria:

The Mebendazole at high concentration level analysis repeatability is assessed using 9 determinations covering the specified range at 3 concentration levels (80.0-120.0%). The analysis repeatability is calculated as % RSD at individual concentration level.

Table 13: Analysis Repeatability: Mebendazole: batch ZR017635PUA721

Concentration Theoretical (%)	q_s (mg)	Area Counts µV.sec	Recovery (%)
80.0 % (1)	80.39	2347049	100.2051
80.0 % (2)	80.37	2365725	101.0276
80.0 % (3)	80.29	2379077	101.6690
Mean:			101.0

RSD (%)	Criterion (%)
0.7	≤ 2.0

Table 14: Analysis Repeatability: Mebendazole: batch ZR017635PUA721

Concentration Theoretical (%)	q_s (mg)	Area Counts µV.sec	Recovery (%)
100.0 % (1)	100.40	2972730	101.6228
100.0 % (2)	100.43	2963285	101.2697
100.0 % (3)	100.30	2963687	101.4147
Mean:			101.4

RSD (%)	Criterion (%)
0.2	≤ 2.0

Table 15: Analysis Repeatability: Mebendazole: batch ZR017635PUA721

Concentration Theoretical (%)	q_s (mg)	Area Counts µV.sec	Recovery (%)
120.0 % (1)	120.54	3497668	99.5904
120.0 % (2)	120.50	3551706	101.1625
120.0 % (3)	120.43	3545434	101.0426
Mean:			100.6

RSD (%)	Criterion (%)
0.9	≤ 2.0 %

Accuracy and Analysis Repeatability at low concentration levels: Test Results and Acceptance Criteria:

The Mebendazole at low concentration levels and the validated impurities analysis repeatability is assessed using 9 determinations covering the specified range at 3 concentration levels: 0.10%, 0.50% and 1.0% %, w/w. Each concentration is prepared 3 times by serial dilutions of 3 separately prepared stock solutions and contains placebo at 100% level. The accuracy is calculated as the % recovery. The analysis repeatability is calculated as % RSD.

Table 16: Accuracy and Analysis Repeatability: At Low Concentration Levels

Concentration Theoretical (%)	Concentration Practical (mg/mL)	Area Counts µV.sec	Recovery (%)	Criteria (%)
0.10 % (1)	0.0001097	3179	109.8098	NA
0.10 % (2)	0.0001077	3123	107.2709	NA
0.10 % (3)	0.0001149	3329	114.4422	NA
Mean recovery:			110.5	80.0-120.0
RSD (%):			3.3	≤ 10.0
0.50 % (1)	0.0005199	15064	104.0840	NA
0.50 % (2)	0.0005185	15023	103.2251	NA
0.50 % (3)	0.0005206	15085	103.6845	NA
Mean recovery:			103.7	90.0-110.0
RSD (%):			0.4	≤ 5.0
1.0 % (1)	0.001024	29672	102.5127	NA
1.0 % (2)	0.001038	30090	103.3641	NA
1.0 % (3)	0.001016	29459	101.2346	NA
Mean recovery:			102.4	90.0-110.0
RSD (%):			1.0	≤ 5.0

NA = Not applicable

Accuracy and Analysis Repeatability (Precision): Impurity(R018986)

Accuracy and Precision: Validated Impurity

Accuracy and Analysis Repeatability: Test Results and Acceptance Criteria

The accuracy and analysis repeatability are assessed using 9 determinations covering the specified range at 3 concentration levels: 0.10, 0.50 and 1.0%, w/w. Each concentration is prepared 3 times by serial dilutions of 3 separately prepared stock solutions and contains placebo and Mebendazole at 100% level.

The impurities present in an un-spiked Mebendazole solution at 100.0% level are taken into account.

The accuracy is calculated as the mean recovery at each concentration level (relative response factor is taken into account). The analysis repeatability is calculated as % RSD.

Table 17: Accuracy and Analysis Repeatability – R018986

Concentration Theoretical (%)		Concentration Practical (mg/mL)	Actual Area Counts µV.sec	Recovery (%)	Criteria (%)
0.10 % (1)		0.0001037	2697	102.2682	NA
0.10 % (2)		0.0001016	2642	100.9940	NA
0.10 % (3)		0.0001030	2679	102.3856	NA
	Mean recovery:			101.9	80.0-120.0
	RSD (%):			0.8	≤ 10.0
0.50 % (1)		0.0005020	13049	98.9552	NA
0.50 % (2)		0.0004970	12919	98.8071	NA
0.50 % (3)		0.0004960	12892	98.5887	NA
	Mean recovery:			98.8	90.0-110.0
	RSD (%):			0.2	≤ 5.0
1.0 % (1)		0.001006	26155	99.1721	NA
1.0 % (2)		0.0009796	25460	97.3757	NA
1.0 % (3)		0.0009894	25715	98.3303	NA
	Mean recovery:			98.3	90.0-110.0
	RSD (%):			0.9	≤ 5.0

NA = Not applicable

Conclusion:

All the results are within acceptance criteria and prove the suitability of the method for an accurate and precise determination of the impurities.

Reproducibility

Reproducibility: Test Results and Acceptance Criteria for Assay

Two analysts (Analyst-1 from Kemwell Lab, India and Analyst-2 from QC- Lab, Lusomedicamenta) analyzed the same 2 representative sample batches 6 times: batch Smarathe-02-027 and batch Smarathe-02-025/03 both spiked with approximately 0.50% of the validated impurity R018986. One un-spiked sample preparation is analysed by each analyst. The analysts used different instruments, different batches of columns and performed the analysis on different days. The reproducibilty is calculated as % RSD of each analyst, the % pooled RSD and % absolute difference between the 2 determinations.

Table 18: Reproducibility: Mebendazole in batch Smarathe-02-027

Date:	19/10/2013			03/11/2013		
HPLC:	ADII/HPLC/04			QC412A		
Column Batch:	B12020 (AD/LC/0433)			B11055 (LC870)		
Analyst	M. Sankar (Analyst-1)			Guinesh Natvarlal (Analyst-2)		
Determination	q_s (mg)	Area Counts µV.sec	Assay (%)	q_s (mg)	Area Counts µV.sec	Assay (%)
Unspiked	200.18	2797977	97.1	202.71	2804444	96.7
1	200.20	2793893	96.9	195.77	2675497	95.5
2	200.42	2804956	97.2	200.62	2792630	97.3
3	200.60	2812518	97.4	200.78	2777521	96.7
4	200.52	2791884	96.7	200.72	2803522	97.6
5	200.26	2799864	97.1	200.20	2774109	96.9
6	200.69	2798108	96.8	201.43	2785450	96.7
Mean:			97.0			96.8

	Result (%)	Criterion (%)
% absolute difference	0.2	≤ 3.0
RSD of analysts	0.3	≤ 2.0
Pooled RSD	0.6	≤ 3.0

G (average weight of the 10 tablets) – Analyst 1: 1000.39mg
G (average weight of the 10 tablets) – Analyst 2: 999.668mg

Table 19: Reproducibility: Mebendazole in batch Smarathe-02-025/03

Date:	20/10/2013			03/11/2013		
HPLC:	ADII/HPLC/04			QC412A		
Column Batch:	B12020 (AD/LC/0433)			B11055 (LC870)		
Analyst	M. Sankar (Analyst-1)			Guinesh Natvarlal (Analyst-2)		
Determination	q_s (mg)	Area Counts µV.sec	Assay (%)	q_s (mg)	Area Counts µV.sec	Assay (%)
Unspiked	199.89	2808481	97.9	200.56	2766438	97.0
1	200.48	2830843	98.4	202.20	2813324	97.9
2	200.41	2833195	98.5	200.85	2789148	97.7
3	200.50	2850919	99.1	200.59	2806909	98.4
4	200.67	2853743	99.1	200.76	2808923	98.4
5	200.46	2836191	98.6	200.43	2799253	98.3
6	200.18	2834703	98.7	200.81	2785401	97.6
Mean:			98.7			98.1

	Result (%)	Criterion (%)
% absolute difference	0.6	≤ 3.0
RSD of analysts	0.3	≤ 2.0
Pooled RSD	0.3	≤ 3.0

G (average weight of the 10 tablets) – Analyst1: 1008.12mg
G (average weight of the 10 tablets) – Analyst2: 1006.102mg

As the reproducibility is incorporated in the method validation the identification of the Mebendazole by HPLC (based on retention times) and the second identification of the Mebendazole by HPLC-PDA are verified by the receiving laboratory (QC Lab, Lusomedicamenta). The retention time windows were evaluated for both batches. The retention time range of the Mebendazole in the sample solution is ±5% of the retention time of the Mebendazole in the first injection of the reference solution. The second identification test is performed on one sample of batch Smarathe-02-07 according to the test method.

Table 20: Identification of Mebendazole with HPLC-PDA

Solution	Maximum	Criteria
Reference solution	234.9 nm	
Sample (batch Smarathe-02-027)	234.9 nm	
Difference between the maximum wavelength of the spectrum of the Mebendazole in the sample solution and the maximum wavelength of the spectrum of the Mebendazole in the reference solution.	0 nm	≤ 2nm

During reproducibility a different terminology was not used for the formulas mentioned in the test method. So Equivalency was not demonstrated and not documented in the lab notebook.

Conclusion:

All the results are within acceptance criteria and prove the suitability of the method for an accurate and precise determination of the API.

Reproducibility: Test Results and Acceptance Criteria for Chromatographic purity

Two analysts (Analyst-1 from Kemwell Lab, India and Analyst-2 from QC- Lab, Lusomedicamenta) analyzed the same 2 representative sample batches 6 times: batch Smarathe-02-027 and batch Smarathe-02-025/03 both spiked with approximately 0.50% of the validated impurity R018986. One un-spiked sample preparation is analysed by each analyst. The analysts used different instruments, different batches of columns and performed the analysis on different days. The reproducibilty is calculated as % RSD of each analyst, % Relative difference and % pooled RSD between the 2 determinations.

For impurities that are spiked to the samples, use the second table.

Table 21: Reproducibility: R018986 in Batch Smarathe-02-027

Date:	19/10/2013			03/11/2013		
HPLC:	ADII/HPLC/04			QC412A		
Column Batch:	B12020 (AD/LC/0433)			B11055 (LC870)		
Analyst	M. Sankar (Analyst-1)			Guinesh Natvarlal (Analyst-2)		
Determination	q_s (mg)	Area Counts µV.sec	% Impurity	q_s (mg)	Area Counts µV.sec	% Impurity
1	200.20	13029	0.4986	195.77	13133	0.4722
2	200.42	13017	0.4982	200.62	13567	0.4889
3	200.60	13003	0.4977	200.78	13706	0.4941
4	200.52	13017	0.4982	200.72	13829	0.4988
5	200.26	13096	0.5012	200.20	13831	0.4989
6	200.69	12981	0.4968	201.43	13720	0.4946
Mean:			0.4985			0.4912
Un-spiked	200.18	653	0.02499	202.71		0.0290

	Result (%)	Criterion (%)
% relative difference	1.5	≤ 20.0
% absolute difference	NA	NA
RSD of analysts	0.3	≤ 5.0
Pooled RSD	1.4	≤ 10.0

q_i (weight impurity in stock solution) – Analyst 1/Analyst 2: 5.063 mg/5.115 mg
Volume impurity stock solution: 200 mL
Dilution factor impurity stock solution: 10000
Volume reference solution: 50 mL
Dilution factor reference solution: 500
%Spiking level: 0.50
NA = Not applicable

Table 22: Reproducibility: R018986 in Batch Smarathe-02-025/03

Date:	20/10/2013			03/11/2013		
HPLC:	ADII/HPLC/04			QC412A		
Column Batch:	B12020 (AD/LC/0433)			B11055 (LC870)		
Analyst	M. Sankar (Analyst-1)			Guinesh Natvarlal (Analyst-2)		
Determination	q_s (mg)	Area Counts µV.sec	% Impurity	q_s (mg)	Area Counts µV.sec	% Impurity
1	200.48	13066	0.4954	202.20	13948	0.5032
2	200.41	12978	0.4921	200.85	13894	0.5011
3	200.50	13071	0.4956	200.59	13720	0.4945
4	200.67	13082	0.4960	200.76	13718	0.4944
5	200.46	12964	0.4915	200.43	13495	0.4860
6	200.18	13044	0.4946	200.81	13642	0.4915
Mean:			0.4942			0.4951
Un-spiked	199.89	708	0.0268	200.56	753	0.0290

	Result (%)	Criterion (%)
% relative difference	0.2	≤ 20.0
% absolute difference	NA	NA
RSD of analysts	0.4	≤ 5.0
Pooled RSD	0.9	≤ 10.0

q_i (weight impurity in stock solution) – Analyst 1/Analyst 2: 5.089 mg/5.115 mg
Volume impurity stock solution: 200 mL
Dilution factor impurity stock solution: 1000
Volume reference solution: 50 mL
Dilution factor reference solution: 500
%Spiking level: 0.50
NA = Not applicable

No unspecified impurities are detected equal to or greater than the reporting threshold of the method (= 0.10 %, w/w).

During reproducibility a different terminology was not used for the formulas mentioned in the test method. Equivalency was not demonstrated and not documented in the lab notebook.

Conclusion:

All the results are within acceptance criteria and prove the suitability of the method for an accurate and precise determination of the impurities.

Detection Limit (DL) and Quantitation Limit (QL)

Detection Limit:

The DL is demonstrated on a solution containing both API and the validated impurities at DL level.

Test results - DL

Table 23: DL values

Compounds	DL value (%)
Mebendazole – R017635	0.01
R018986	0.003

Quantitation Limit:

Test Results and Acceptance Criteria

The QL value is set equal to the reporting threshold value and is supported by the accuracy and analysis repeatability at RT level.

Table 24: QL values

Compounds	Criteria	QL value (%)
Mebendazole – R017635	See 0	0.02
R018986	See 0	0.01

Conclusion:

The quantitation limits are reported. The accuracy and analysis repeatability at the RT level proves an accurate and precise determination of impurities at the lower concentration level.

Reporting Threshold

Reporting Threshold:

The reporting threshold is 0.10%, w/w. Accuracy and analysis repeatability are demonstrated at this level.

Linearity

Linearity: Test Results and Acceptance Criteria

For the Mebendazole the linearity is demonstrated on the following concentrations:

0.10 – 0.50 – 1.0 – 5.0 – 25.0 – 80.0 – 90.0 – 100.0 – 110.0 – 120.0%, w/w.

For the validated impurity the linearity is demonstrated on the following concentrations: 0.10 – 0.20 – 0.50 – 0.80 – 1.0%, w/w.

Linearity is evaluated by visual inspection of a plot and mathematical estimates of the degree of linearity.

Table 25: Linearity

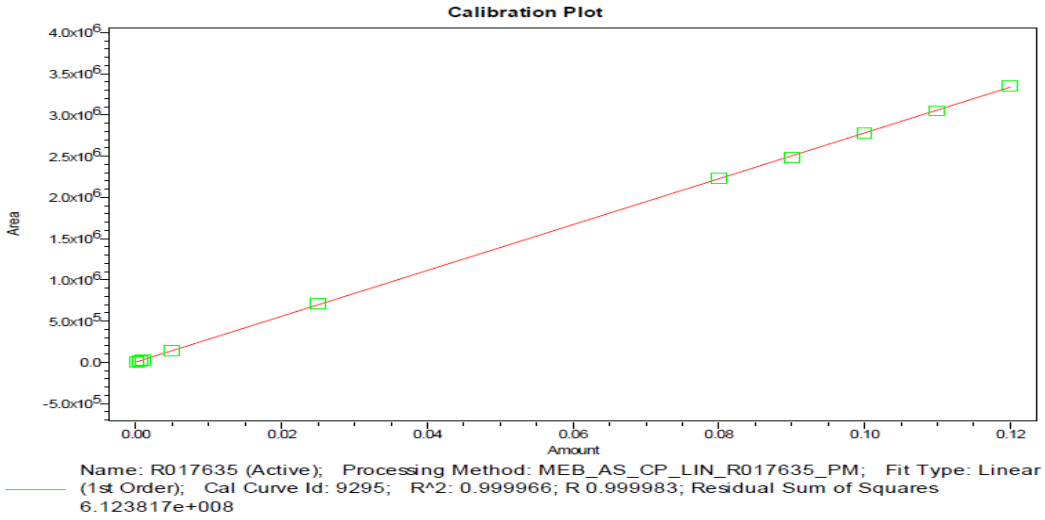
Reference	r	Criteria	% RSD*	Criteria (%)	Visual
R018986: 0.10% - 1.0%	0.999	≥ 0.99	2.3	≤ 15.0	Linear
Mebendazole: 80.0% - 120.0%	0.999	≥ 0.999	0.4	≤ 3.0	Linear
Mebendazole: 0.10% - 120.0%	0.999	≥ 0.99	5.6	≤ 10.0	Linear

*: RSD on response factors

Table 26: Linearity Mebendazole (0.10 % - 120.0 %)

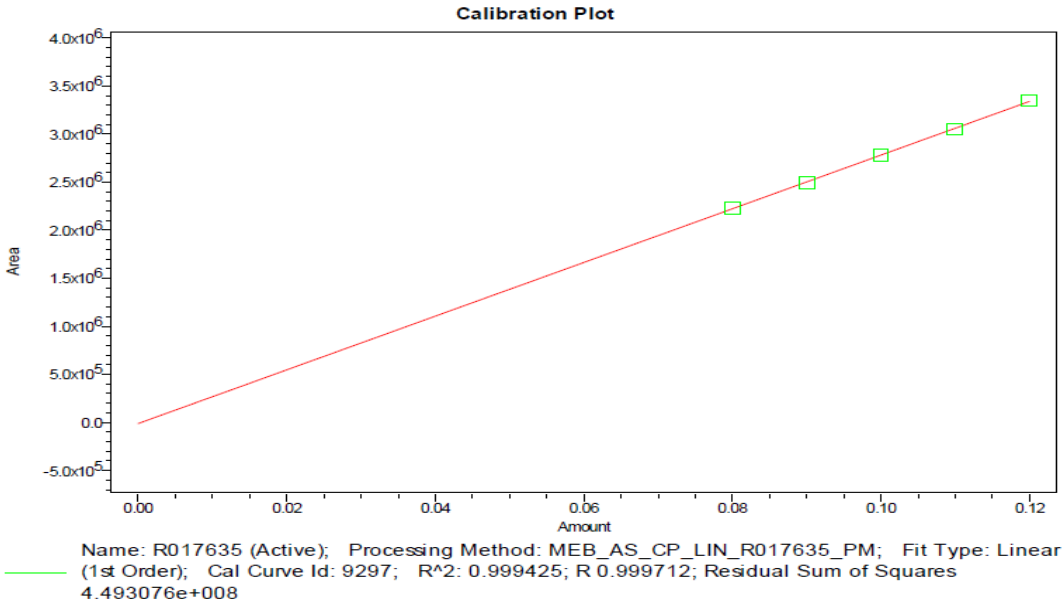
Concentration API Theoretical (%)	Concentration API Practical (mg/mL)	Actual Area Counts µV.sec	Response Factor (Area Counts / Concentration mg/mL)
0.10	0.0001000	3290	32900000.0000
0.50	0.0004999	13987	27979595.9191
1.0	0.0009998	27905	27910582.1164
5.0	0.0049990	140344	28074414.8829
25.0	0.0249948	708526	28346936.1627
80.0	0.0799833	2233098	27919553.2067
90.0	0.0899812	2489529	27667212.7066
100.0	0.0999792	2784319	27848982.5883
110.0	0.1099771	3051219	27744130.3689
120.0	0.1199750	3349328	27916882.6838

Fig 11: Linearity Mebendazole (0.10% - 120.0%)



Y-axis	: Area counts in µV.sec
X-axis	: Concentration in mg/ml
Slope	: 27806214.1462
Intercept	: 2283.9708
Residual Sum of Squares	: 612381728.8841

Fig 12: Linearity Mebendazole (80.0% - 120.0%)

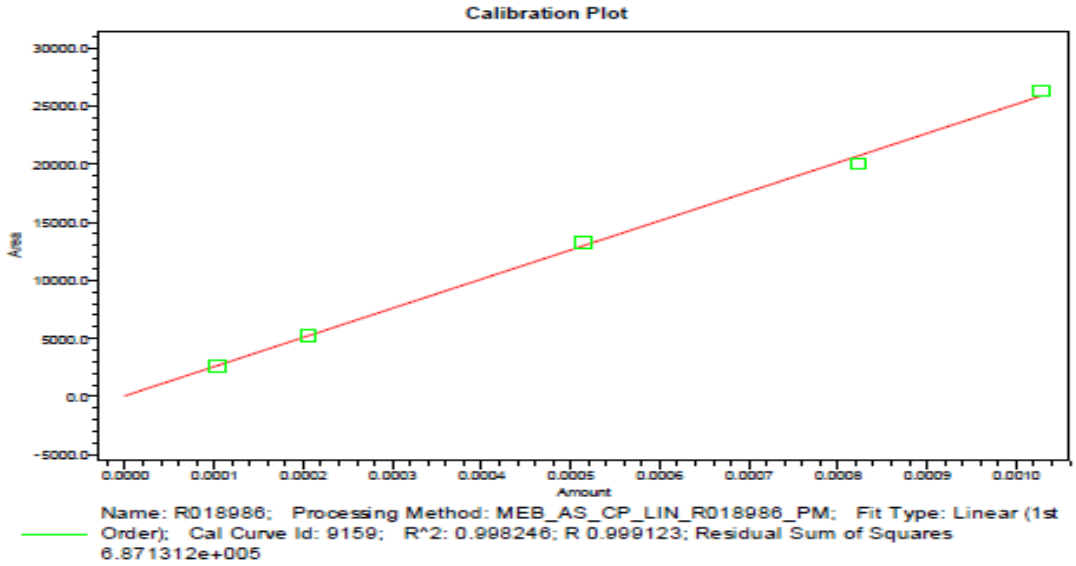


Y-axis	: Area counts in µV.sec
X-axis	: Concentration in mg/ml
Slope	: 27947285.3005
Intercept	: -12647.5086
Residual Sum of Squares	: 449307614.8291

Table 27: Linearity validated impurity R018986 (0.10% - 1.0%)

Concentration Theoretical (%)	Concentration Practical (mg/mL)	Area Counts µV.sec	Response Factor (Area Counts / Concentration mg/mL)
0.10	0.0001029	2648	25733722.0602
0.20	0.0002057	5236	25454545.4545
0.50	0.0005143	13230	25724285.4365
0.80	0.0008229	20032	24343176.5706
1.0	0.0010286	26269	25538596.1501

Fig 13: Linear graph R018986 (0.10% - 1.0%)



Y-axis : Area counts in µV.sec

X-axis : Concentration in mg/ml

Slope : 25075207.9206

Intercept : 70.7728

Residual Sum of Squares : 687131.2320

Conclusion:

All acceptance criteria are met demonstrating the linearity of the method.

Range

Range: Test Results and Acceptance Criteria

The range of the test method describes the interval between the upper and lower concentration for which it has been demonstrated that the test method has a suitable level of accuracy, precision and linearity.

Test Results and Acceptance Criteria: Range

Table 28: Range

Compounds	Target Range
Mebendazole	0.10 % - 120.0%
R018986	0.10 % - 1.0 %

Robustness

Robustness: Test Results and Acceptance Criteria

The robustness of the chromatographic conditions is tested by varying the following parameters: column temperature (± 5°C), flow (± 0.20 ml/min), start of gradient (± 1 unit), concentration of mobile phase A (± 0.0025% TFA), based on the development data Experiment -11, 12, 13 and 14 are worst case experiments percentage organic modifier in the mobile phase at the start of the gradient.

Table 29: Robustness

Parameter	Resolution (R_s) R019020-R017635	Tailing Factor (T) Mebendazole
Nominal	6.4	1.0
Flow rate 1.3mL/min	6.6	1.0
Flow rate 1.7mL/min	6.4	1.0
Column Lot: B	6.5	1.0
Column temperature 35°C	6.2	1.0
Column temperature 45 °C	7.4	1.0
Gradient : -1unit	6.7	1.0
Gradient : +1unit	6.5	1.0
Mobile phase TFA 0.0225%	7.5	1.0
Mobile phase TFA 0.0275%	6.9	1.0
Exp. No: 11	7.4	1.0
Exp. No: 12	8.2	1.0
Exp. No: 13	5.9	1.0
Exp. No: 14	6.7	1.0

The robustness of the sample preparation is tested by varying the following parameters: the way of sonication time variation (± 5 minutes), by addition of formic acid volume (±5 mL) of the sample preparation.

Table 30: Robustness sample preparation (Sample: batch Smarathe-02-027)

Parameter	G (mg)	q_s (mg)	Area Counts µV.sec	Assay (%)	%Absolute Difference
Sample preparation technique (Nominal)	999.81	200.29	2777034	97.6	NA
Sonication time: 15minutes	999.81	200.25	2762804	97.1	0.5
Sonication time: 25minutes	999.81	200.53	2788114	97.9	0.3
Formic acid: 25mL	999.81	200.42	2766037	97.2	0.4
Formic acid: 35mL	999.81	200.07	2774776	97.7	0.1
				Criterion:	≤ 3.0%

NA = Not applicable
G = average weight of the 10 tablets

Conclusion:

The worst case Resolution of 2.5 is set as minimum system suitability acceptance criterion. A worst case experiment (low column temperature in combination with low flow and less concentration of TFA) was set up to determine this acceptance criterion.

A Limit of < 2.0 is set as system suitability solution acceptance ctiterion for the Tailing factor.

The Assay values of the different sample preparations are all within 3.0% absolute difference compared to the nominal value.

The robustness of the method is demonstrated by proving the validity of the method after small deliberate changes to the method parameters.

Stability of Solutions

Stability of solutions: Test Results and Acceptance Criteria

The stability of the reference solution, un-spiked sample and spiked sample solution (spiked with the validated impurities) is determined during a period of 7 days. The solutions are stored in amber glassware at ambient conditions on table top and at 2-8°C condition.

Test Results and Acceptance Criteria

Table 311: Stability of Solutions

Solution		t = 0		t = 1 day (Table Top)		Rel. Diff. (%)	Criteria (%)
Reference	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	2836245	99.7	0.3	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2786958	97.8	0.3	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	1174	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	13881	0.54	1.9	≤ 15.0
No New degradation products at t = 1 days							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 322: Stability of Solutions (continued)

Solution		t = 0		t = 1 day (2-8°C)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	2835582	99.7	0.3	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2787588	97.8	0.3	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	769	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	13466	0.53	0.0	≤ 15.0
No New degradation products at t = 1 days							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 333: Stability of Solutions (continued)

Solution		t = 0		t = 2 day (Table Top)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	3019272	105.0	5.0	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2808073	97.5	0.6	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	1802	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	14776	0.57	7.5	≤ 15.0
No New degradation products at t = 2 days							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 344: Stability of Solutions (continued)

Solution		t = 0		t = 2 day (2-8°C)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	2860372	99.5	0.5	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2806316	97.4	0.7	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	921	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	13773	0.53	0.0	≤ 15.0
No New degradation products at t = 2 days							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 35: Stability of Solutions (continued)

Solution		t = 0		t = 3 day (Table Top)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	NA	NA	NA	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2786199	97.3	0.8	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	1784	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	14680	0.57	7.5	≤ 15.0
New degradation products at t = 3 days: < RT							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 36: Stability of Solutions (continued)

Solution		t = 0		t = 3 day (2-8°C)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	2842335	99.5	0.5	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2783167	97.2	0.9	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	775	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	13654	0.53	0.0	≤ 15.0
New degradation products at t = 3 days: < RT							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 375: Stability of Solutions (continued)

Solution		t = 0		t = 5 day (Table Top)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	NA	NA	NA	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2805670	98.5	0.4	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	2334	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	15323	0.60	13.2	≤ 15.0
New degradation products at t = 5 days: < RT							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 386: Stability of Solutions (continued)

Solution	t = 0			t = 5 day (2-8°C)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	2842827	100.0	0.0	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2778290	97.6	0.5	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	816	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	13503	0.53	0.0	≤ 15.0
New degradation products at t = 5 days: < RT							Pass

G (average weight of the 10 tablets for sample solution): 999.81mg

Table 397: Stability of Solutions (continued)

Solution	t = 0			t = 7 day (Table Top)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	NA	NA	NA	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2877533	99.9	1.8	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	3643	0.14	> RT	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	16823	0.65	22.6	≤ 15.0
New degradation products at t = 7 days: < RT							Pass

G (average weight of the 10 tablets for Sample Solution): 999.81mg

Table 40: Stability of Solutions (continued)

Solution		t = 0		t = 7 day (2-8°C)		Rel. Diff. (%)	Criteria (%)
Reference Solution	Conc. (mg/ml)	Area counts µV.sec	Recovery (%)	Area counts µV.sec	Recovery (%)		
Reference solution 100% level	0.10004	2824960	100.0	2874740	100.0	0.0	≤ 2.0
Assay - unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Assay (%)	Area counts	Assay (%)	Rel. Diff. (%)	Criteria (%)
Mebendazole	200.42	2776662	98.1	2830868	98.3	0.2	≤ 2.0
Degradation products unspiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.42	747	< RT	1147	< RT	NA	≤ 20.0
Degradation products spiked sample solution (batch Smarathe-02-027)	q_s (mg)	Area counts	Impurity (%)	Area counts	Impurity (%)	Rel. Diff. (%)	Criteria (%)
R018986	200.39	13421	0.53	14066	0.54	1.9	≤ 15.0
New degradation products at t = 7 days: < RT							Pass

G (average weight of the 10 tablets for Sample Solution): 999.81mg

Conclusion:

Acceptance solution stability has been demonstrated for reference solution at 100% level, sample solution unspiked and sample solution spiked with the validated impurity at 0.50% level during a period of 7 days when stored in amber glassware at 2-8°C condition

Solution stability of reference solution at 100% level were not meeting the acceptance criteria on table top at 2nd day.Hence the reference solution at 100% level is stable for one day.

Sample solution unspiked and sample solution spiked with the validated impirity at 0.50% level at ambient conditions were not meeting the acceptance criteria on table top at 7th day. Hence sample solution unspiked and sample solution spiked with the validated impirity at 0.50% level is stable upto 5 days.

Filtration Study

Stability of solutions: Test Results and Acceptance Criteria

Filtrates collected from the sample solution (spiked with approximately 0.50% validated impurities) and a blank are analyzed and compared with centrifuged (unfiltered) samples of the same solutions.

Table 41: Filtration study

Spiked Sample Solution (batch Smarathe-02-027)		Filtered		Centrifuged		Rel. Diff. (%)	Criteria (%)
	q_s (mg)	Area counts µV.sec	Assay (%)	Area counts µV.sec	Assay (%)		
Mebendazole	200.20	2791483	96.8	2793699	96.9	0.1	≤ 2.0
	q_s (mg)	Actual Area counts µV.sec	Impurity (%)	Area counts µV.sec	Impurity (%)		
R018986	5.063	12975	0.4966	13065	0.5000	0.7	≤ 15.0
Contaminants must be smaller than 0.10%							Pass

G (average weight of the 10 tablets for Sample Solution): 1000.39mg

Conclusion:

The filtration study demonstrates the absence of interfering contaminants extracted from the filter and the absence of significant adsorbance to the filter bed.

Relative Response Factors

Stability of solutions: Test Results

The Relative Response Factors (RRF) are calculated from the slopes of the calibration curves of the validated impurities in the specified range and the API in the assay range.

$$RRF = \frac{Slope_{Impurity\ (specified\ range)}}{Slope_{API\ (assay\ range)}}$$

Table 428: Calculation of RRF values

Compound	Range	Slopes	RRF
Mebendazole	80.0 – 120.0%	27947285.3005	-
R018986	0.10 – 1.0%	25075207.9206	0.897

Forced degradation Study

A forced degradation study was conducted to demonstrate that the method is stability indicating. Separate portions of drug product, drug substance and placebo were exposed to following stress condition to induce degradation.

Acid Stress: (For sample)

1) Samples were stressed with 5mL of 1N HCl at 80°C for 2 hours, neutralized with 5ml of 1N NaOH.
(% degradation was not achieved)

2) Samples were stressed with 10mL of 1N HCl at 80°C for 3 hours, neutralized with 10ml of 1N NaOH. (% degradation was not achieved)

3) Samples were stressed with 5mL of 5N HCl at 80°C for 2 hours, neutralized with 5ml of 5N NaOH.
(% degradation was achieved)

Base Stress: (For sample)

1) Samples were stressed with 2.5mL of 0.5N NaOH at 80°C for 2 hours, neutralized with 2.5ml of 0.5N HCl. (% degradation was not achieved)
2) Samples were stressed with 5mL of 0.5N NaOH at 75°C for 45mins, neutralized with 5ml of 0.5N HCl. (% degradation was not achieved)

Oxidative Stress: (For sample)

1) Samples were stressed with 5mL of 1% hydrogen peroxide (H2O2) at 80°C for 1 hour.
(% degradation was not achieved).

2) Samples were stressed with 5mL of 3% hydrogen peroxide (H2O2) at 70°C for 1 hour.
(% degradation was not achieved).

3) Samples were stressed with 5mL of 3% hydrogen peroxide (H2O2) at 80°C for 50mins.
(% degradation was not achieved).

Thermal Stress: (For sample)

Samples were exposed to heat at 80°C for about 72hrs.

Photolytic stress: (For sample)

Photolytic degradation study was carried out by exposing the samples to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours / square meter. (Neutronic photo stability chamber - by using light source option 2)

Stressed samples were injected into the HPLC system with photo diode array detector by following methodology section given in the protocol.

No Interference was observed from diluent and placebo peaks at the retention time of Mebendazole peak.

The chromatograms of the stressed test samples were evaluated for peak purity of Mebendazole peak using Waters Empower networking software. For all forced degradation test solutions, the purity angle is less than the purity threshold for the Mebendazole peak.

The results obtained are given in Table 5 and Table 6.

Results obtained from forced degradation studies for sample 500mg strength are summarised in Table 43.

Table 43:

Mode of degradation	Condition	% Degradation	Purity angle	Purity threshold	Purity Flag
Unstressed Test sample	Unstress	-	0.091	0.257	No
Thermal	80°C for 72 hours	0.06	0.089	0.267	No
Photolytic	1.2million LUX Hours & 200 watt hours/square meter	0.05	0.086	0.270	No
Acid	5N HCl / 2 hours heating at 80°C	14.5	0.069	0.300	No

Base	0.5N NaOH / 45mins heating at 70°C	2.6	0.107	1.990	No
Oxidation	3%H2O2 / 50mins heating at 80°C	4.2	0.102	0.282	No

No peaks were detected at the retention time of the related substances and Mebendazole peak in the chromatogram of the Diluent / mobile phase and placebo solutions.

Peak purity angle was less than Peak purity threshold for Mebendazole peak in stressed test solution.

There was no tick mark in the purity flag column. Method is specific and stability indicating.

Observation:

1. Blank solution, one peak was eluted at 4.1minutes.
2. No peak was eluted in placebo solution (Unstress)

Results obtained from forced degradation studies for Active Pharmaceutical Ingredient are summarised in Table 44.

Table 44:

Mode of degradation	**Condition**	**% Degradation**	**Purity angle**	**Purity threshold**	**Purity Flag**
Unstressed API	Un stress	-	0.085	0.250	No
Thermal	80°C for 72 hours	0.03	0.077	0.252	No
Photolytic	1.2million LUX Hours & 200 watt hours/square meter	0.41	0.069	0.254	No
Acid	5N HCl / 2 hours heating at 80°C	16.5	0.060	0.328	No
Base	0.5N NaOH / 45mins heating at 70°C	1.37	0.092	0.299	No
Oxidation	3%H2O2 / 50mins heating at 80°C	45.2	0.078	0.258	No

No peaks were detected at the retention time of the related substances and Mebendazole peak in the chromatogram of the Diluent / mobile phase and placebo solutions.

Peak purity angle was less than Peak purity threshold for Mebendazole peak in stressed test solution.

There was no tick mark in the purity flag column. Method is specific and stability indicating.

Observation:

1. Blank peak was eluted at RT about 4.1minutes.
2. Acid stress condition unknown impurity at RT 22.347minutes were increased. Eighteen new peaks were observed in acid stress condition (5N HCl).

Conclusion

The results prove that the method is stability indicating of the Mebendazole.

System Suitability Tests

Stability of solutions: Test Results and Acceptance Criteria

The system suitability tests results generated during method validation are listed and evaluated

Table 459: System Suitability tests results

Date	Validation Charac-teristic	Result set ID	mg / mL Reference Solution 1	Mean Area Count µV.sec (n = 5)	RT Area Count µV.sec	Recovery RT 80.0-120.0%	mg / mL Reference Solution 2	Area Count µV.sec Reference Solution 2	Recovery Ref Sol 2 98.0-102.0%	Area Count µV.sec Control Reference	Recovery Control Reference 98.0-102.0%
03/06/2013	Specificity	NA	0.09994	2838964	3099	109.2	0.10002	2868110	100.9	2869913	101.1
05/06/2013	STS-0 day	NA	0.09986	2819491	2938	104.2	0.09982	2829735	100.4	2830630	100.4
05/06/2013	Linearity	NA	0.09986	2847073	3057	107.4	0.09982	2887172	101.4	2874811	101.0
05/06/2013	RB-Spl preparation Variable	NA	0.09986	2835876	3038	107.1	0.09982	2829324	99.8	2844750	100.3
06/06/2013	STS-1 day	NA	0.09972	2834965	2951	104.1	0.09976	2840990	100.2	2840879	100.2
06/06/2013	RB-CHP-Unchanged	NA	0.09972	2921907	3024	103.5	0.09976	2950702	100.9	2929883	100.3
06/06/2013	RB-Flow-CHP-1.3mL/min	NA	0.09972	3383815	3645	107.7	0.09976	3396476	100.3	3371918	99.6
06/06/2013	RB-Flow-CHP-1.7mL/min	NA	0.09972	2562919	2725	106.3	0.09976	2559340	99.8	2566937	100.2
07/06/2013	STS-2 day	NA	0.09986	2870824	3000	104.5	0.09996	2869904	99.9	2873723	100.1
08/06/2013	STS-3 day	NA	0.09980	2849959	3322	116.6	0.09982	2849337	100.0	2862286	100.4
08/06/2013	RB-CHP-Column temp. 35°C	NA	0.09986	2880316	3097	107.5	0.09996	2891394	100.3	2878343	99.9
08/06/2013	RB-CHP-Column temp. 45°C	NA	0.09986	2881461	3024	104.9	0.09996	2876529	99.7	2872755	99.7

RB – Robustness, CP – Chrmatographic purity, CHP – Chromatographic parameter, NA – Not applicable, STS – Stability of solution

Table 46: System Suitability tests results

Date	Validation Charac-teristic	Result set ID	mg / mL Reference Solution 1	Mean Area Count µV.sec (n = 5)	RT Area Count µV.sec	Recovery RT 80.0-120.0%	mg / mL Reference Solution 2	Area Count µV.sec Reference Solution 2	Recovery Ref Sol 2 98.0-102.0%	Area Count µV.sec Control Reference	Recovery Control Reference 98.0-102.0%
08/06/2013	RB-CHP-Column-B	NA	0.09980	2950628	3359	113.8	0.09982	2932306	99.4	2963640	100.4
08/06/2013	RB-CHP-Gradient: -1 unit	NA	0.09980	2976542	3369	113.2	0.09982	2971965	99.8	2982542	100.2
08/06/2013	RB-CHP-Gradient: +1 unit	NA	0.09980	2994270	3516	117.4	0.09982	3000111	100.2	2998984	100.2
10/06/2013	STS-5 day	NA	0.09980	2834882	3258	114.9	0.09974	2837975	100.2	2840659	100.2
11/06/2013	RB-CHP-TFA 0.0225%	NA	0.09992	2916754	3261	111.8	0.09996	2913261	99.8	2906015	99.6
11/06/2013	RB-CHP-TFA 0.0275%	NA	0.09992	2879368	3433	119.2	0.09996	2894881	100.5	2854142	99.1
11/06/2013	RB-CHP-Exp.No:12	NA	0.09992	3328748	3681	110.6	0.09996	3321165	99.7	3331008	100.1
11/06/2013	RB-CHP-Exp.No:14	NA	0.09996	3340088	3552	106.3	0.09996	3325950	99.5	3345493	100.2
12/06/2013	STS-7 day	NA	0.09992	2871667	2884	100.4	0.09998	2888148	100.5	2874399	100.1
15/06/2013	RB-CHP-Exp.No:11	NA	0.10000	3311713	3659	110.5	0.10046	3326641	100.0	3317909	100.2
15/06/2013	RB-CHP-Exp.No:13	NA	0.10000	3320981	3658	110.1	0.10046	3334616	100.0	3318279	99.9
17/06/2013	Accuracy-Assay	NA	0.09968	2913020	2933	100.7	2952629	0.09974	101.3	2915687	100.1

RB – Robustness, CP – Chrmatographic purity, CHP – Chromatographic parameter, NA – Not applicable, STS – Stability of solution

Table 47: System Suitability tests results

Date	Validation Charac-teristic	Result set ID	mg / mL Reference Solution 1	Mean Area Count µV.sec (n = 5)	RT Area Count µV.sec	Recovery RT 80.0-120.0%	mg / mL Reference Solution 2	Area Count µV.sec Reference Solution 2	Recovery Ref Sol 2 98.0-102.0%	Area Count µV.sec Control Reference	Recovery Control Reference 98.0-102.0%
18/06/2013	Accuracy - CP	NA	0.10014	2901423	2881	99.3	0.10020	2898106	99.8	2919667	100.6
19/06/2013	Reproducib ility: batch Smarathe-02-027	NA	0.10030	2887844	3282	113.6	0.10040	2893031	100.1	2888719	100.0
19/06/2013	Filter study	NA	0.10030	2887844	3282	113.6	0.10040	2893031	100.1	2889145	100.0
20/06/2013	Quantitatio n Limit	NA	0.09994	2801491	2860	102.1	0.09984	2800890	100.1	2800711	100.0
20/06/2013	Reproducib ility: batch Smarathe-02-025/03	NA	0.09994	2889659	2753	95.3	0.09984	2886705	100.0	2898616	100.3
21/06/2013	Linearity	NA	0.09970	2759574	2993	108.5	0.09976	2759310	99.9	2789781	101.1

RB – Robustness, CP – Chrmatographic purity, CHP – Chromatographic parameter, NA – Not applicable, STS – Stability of solution

Table 48: System Suitability tests results (continued)

Date	Validation Charac-teristic	Result set ID	Resolution $R_s^{**} \geq 2.5$	Tailing Factor** < 2.0	%RSD* (n = 5) ≤ 1.0%
03/06/2013	Specificity	NA	6.3	1.0	0.7
05/06/2013	STS-0 day	NA	6.4	1.0	0.4
05/06/2013	Linearity	NA	6.5	1.0	0.2
05/06/2013	RB-Spl preparation Variable	NA	6.8	1.0	0.1
06/06/2013	STS-1 day	NA	6.4	1.0	0.1
06/06/2013	RB-CHP-Unchanged	NA	6.4	1.0	1.0
06/06/2013	RB-Flow-CHP-1.3mL/min	NA	6.6	1.0	0.5
06/06/2013	RB-Flow-CHP-1.7mL/min	NA	6.4	1.0	0.2
07/06/2013	STS-2 day	NA	7.0	1.0	0.1
08/06/2013	STS-3 day	NA	6.3	1.0	0.1
08/06/2013	RB-CHP-Column temp. 35°C	NA	6.2	1.0	0.1
08/06/2013	RB-CHP-Column temp. 45°C	NA	7.4	1.0	0.1

*: Relative Standard Deviation for the areas of the 5 successive injections of Reference Solution 1

**: $R_s - A_s$ at begin of each sequence

Table 4910: System Suitability tests results (continued)

Date	Validation Charac-teristic	Result set ID	Resolution $R_s^{**} \geq 2.5$	Tailing Factor** < 2.0	%RSD* (n = 5) ≤ 1.0%
08/06/2013	RB-CHP-Column-B	NA	6.5	1.0	0.3
08/06/2013	RB-CHP-Gradient: -1 unit	NA	6.7	1.0	0.1
08/06/2013	RB-CHP-Gradient: +1 unit	NA	6.5	1.0	0.1
10/06/2013	STS-5 day	NA	6.3	1.0	0.1
11/06/2013	RB-CHP-TFA 0.0225%	NA	7.5	1.0	0.2
11/06/2013	RB-CHP-TFA 0.0275%	NA	6.9	1.0	1.0
11/06/2013	RB-CHP-Exp.No:12	NA	8.2	1.0	0.1
11/06/2013	RB-CHP-Exp.No:14	NA	6.7	1.0	0.1
12/06/2013	STS-7 day	NA	7.3	1.0	0.1
15/06/2013	RB-CHP-Exp.No:11	NA	7.4	1.0	0.1
15/06/2013	RB-CHP-Exp.No:13	NA	5.9	1.0	0.1
17/06/2013	Accuracy-Assay	NA	7.2	1.0	0.6

*: Relative Standard Deviation for the areas of the 5 successive injections of Reference Solution 1

**: $R_s - A_s$ at begin of each sequence

Table 5011: System Suitability tests results (continued)

Date	Validation Charac-teristic	Result set ID	Resolution R_s ** ≥ 2.5	Tailing Factor** < 2.0	%RSD* (n = 5) ≤ 1.0%
18/06/2013	Accuracy - CP	NA	7.1	1.0	0.2
19/06/2013	Reproducibility: batch Smarathe-02-027	NA	6.6	1.0	0.1
19/06/2013	Filter study	NA	6.6	1.0	0.1
20/06/2013	Quantitation Limit	NA	7.1	1.0	0.1
20/06/2013	Reproducibility: batch Smarathe-02-025/03	NA	6.6	1.0	0.1
21/06/2013	Linearity	NA	7.0	1.0	0.2

*: Relative Standard Deviation for the areas of the 5 successive injections of Reference Solution 1

**: $R_s - A_s$ at begin of each sequence

CONCLUSION:

This method validation report describes the results and acceptance criteria for the validation of DS-TMD-12345, Version: 1.0.

Since all the acceptance criteria have been met the report demonstrates that the test method is suitable for identification and assay of R017635 and determination of its impurities in R017635 G002, Mebendazole Chewable 500mg Tablets.

RESULTS AND DISCUSSION

The objective of the proposed work was to develop a simple, reliable method for the determination of Mebendazole to validate the methods according to USP and ICH Guidelines Q2(R1) and applying the same for its estimation in laboratory prepared mixtures.

In this method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate the title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time, retention time and resolution. The system with Phosphate buffer: Methanol (pH 2.5±0.1) (70:30 v/v) with 0.8ml.min^{-1} flow rate is quite healthy.

The optimum wavelength for detection was 254nm at which better detector response for the title drug was obtained. The retention time for was found to be 4.1min. The calibration was linear in concentration range of 1-10 µg mL^{-1} withregression 0.999, intercept +2056 and slope 18593.

Sample to sample precision and accuracy were evaluated using six samples of one concentration, which were prepared and analyzed on same day. Day to day variability was assessed using oneconcentration analyzed on two different days. These results show the precision and accuracy of the assay based on % R.S.D.(0.82 – 0.96%) reported was found to be less than 2% and recovery (99.9 – 100.25%) were found to be in the range of 98 – 102%.The proposed method was validated in

accordance with ICH parameters and the applied for analysis of the same in laboratory prepared mixtures.

Thus the proposed method is NEW, accurate, simple, rapid and selective for the estimation of Mebendazolein laboratory prepared mixtures.Hence, these methods can be conveniently adopted for the routine analysis of Mebendazole formulation in quality control laboratories.

SUMMARY

Summary:

On the basis of the experiment, I can conclude that the RP-HPLC method developed for the Estimation of Mebendazolecan be used for routine analysis Quality Control (QC) Samples.

Mebendazoleis determined by reverse phase HPLC using,

1. Column: Zorbax SB C18 (150×4.6) mm, id 5.0µm particle size or equivalent
2. Column Temperature : 40°C ± 2.0°C
3. Auto-sampler Temperature : 5°C
4. Flow rate : 1.50 mL/min
5. Injection volume : 10 µL
6. Detection : UV at 250 nm
7. Run time : 35 minutes
8. Elution mode : Gradient elution

   Buffer 0.025% Trifluoro Acetic Acid: Acetonitrile combination as mobile phase in gradient programme.

   A linear gradient is programmed as described in the following table;

Time(min.)	0	20	29	30	35
% A	90	70	30	90	90
% B	10	30	70	10	10

After development of the method, it was validated for system suitability, specificity and linearity, limit of detection and limit of quantification, precision, and accuracy.

- ✓ The system suitability was found to be within the limits. The percent relative standard deviation (% RSD) for the area for Mebendazole of five

replicateinjections of the Reference Solution 1 should be less than or equal to 2.0 %. The per cent relative standarddeviation (% RSD) for the area for Mebendazole is 0.2.

- ✓ The percentage recovery of Mebendazole in Reference Solution 2is 98.0% ≤ % Recovery ≤ 102.0%. The percentage recovery of Mebendazole is 98.6.
- ✓ The tailing factor (T) for the Mebendazole peak from first replicate of reference solution must be less than 2.0, as calculated by the current USP method. The tailing factor (T) for the Mebendazole peak is 1.0.
- ✓ The Resolution factor (R) between (R019020 and R017635) in the selectivity solution must be greater than 2.5 calculated by current USP method. The Resolution factor (R) between (R019020 and R017635) is 4.6.
- ✓ The reference solution must be injected as a calibration check after each series of maximum 12 sample injections and after the last sample injection. The percentage Recovery of Mebendazole is 98.0% ≤ % Recovery ≤ 102.0%. The percentage Recovery of Mebendazole is 98.6.
- ✓ The limit were Not more than RSD <2%. The retention time of Mebendazole is 17.3mins.
- ✓ The precision was found to be within the limits. The limit were not more than RSD <2%. This indicates that the method is precise. The data regarding the precision are shown in Table no 8 and 9.
- ✓ From the linearity table, it was found that, the drug obeys Beer's Law. For HPLC the calibration plot of Mebendazole was observed as linear in the range 0.1-120 mg/mL and the correlation coefficient were found to be 0.999.
- ✓ From the results shown in the accuracy table and, it was found thatrecovery value of pure drug from the solution were between 98%to102% Which indicates that the method is accurate.

Table 51: Summary of the present study (RP-HPLC)

Validation Parameters	Mebendazole (R017635)
Mobile phase	0.025% Trifluoro acetic Acid:ACN(90:10, v/v) (In gradient programme)
Flow rate	1.5 mL/min
Detection Wavelength	250 nm
Retention Time	About 17 minutes
Run Time	35 min
USP Resolution factor	4.6
LOD for Mebendazole	0.01%
LOD for R018986	0.003%
LOQ for Mebendazole	0.02%
LOQ for R018986	0.01%
Linearity for Mebendazole	0.10-120.0%, R=0.999
Linearity for Mebendazole	80.0-120.0%, R=0.999
Linearity for R018986	0.10-1.0%, R=0.999
Precision for batch: smarathe-02-027	
Precision for Mebendazole	0.3% (%RSD ≤ 2.0)
Precision for R018986	0.3% (%RSD ≤ 5.0)
Precision for batch: smarathe-02-025/03	
Precision for Mebendazole	0.3% (%RSD ≤ 2.0)
Precision for R018986.	0.4% (%RSD ≤ 5.0)

Recovery (Accuracy)

Accuracy Mebendazole 80.0%	- 100.2, 101.0, 101.7 (97.0-103.0%) -101.0 (98.0-102.0)
Accuracy Mebendazole 100.0%	- 101.6, 101.3, 101.4 (99.7-103.0%) -101.4 (98.0-102.0)
Accuracy Mebendazole 120.0%	- 99.6, 101.2, 101.0 (99.7-103.0%) -100.6 (98.0-102.0)
Accuracy Mebendazole0.10%	-110.5 (80.0-120.0)
Accuracy Mebendazole 0.50%	-101.0 (90.0-110.0)
Accuracy Mebendazole 0.10%	-101.0 (90.0-110.0)
Accuracy R018986 0.10%	-101.9 (80.0-120.0)
Accuracy R018986 0.50%	-98.8 (90.0-110.0)
Accuracy R018986 0.10%	-98.3 (90.0-110.0)
Mebendazole Condition	- Stable for 7 days in BT
Range	- 0.10% 120.0%

CONCLUSION

- The proposed method was found to be simple, precise, accurate and rapid for determination of Mebendazole, in pure form.
- The mobile phase is simple to prepare and economical.
- The sample recoveries in all formulations were in good agreement within the limit.
- Hence, this method can be easily and conveniently adopted for routine analysis of Mebendazole.

BIBLIOGRAPHY

1. Takeruhiguchi, Einarbrochmann, Hamffenhanssen. Text book of pharmaceutical analysis.New Delhi: CBS publishers and distributors; 2005. p. 1-2.
2. Sharma BK. Instrumental methods of Chemical Analysis. 19th ed. Meerut: GoelPublishing House; 2000. p. 1-2.
3. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi: CBS Publishers and Distributors; 1997. p. 2,1,52,296-305.
4. G.H.Jeffery. J.Bassett. J.Mendham. R.C.Denney. vogel's text book of quantitative chemical analysis. 5th ed. Singapore: ELBS with longman; p. 2-4.
5. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd ed. New Delhi: CBS Publishers and Distributors; 1986. p. 1,20.
6. Willard HH, Merritt LL, Dean JJA, Frank AS. Instrumental Method of Analysis. 7thed. New Delhi: CBS Publishers and Distributors; 1986. p. 1-4.
7. Kaur H. Spectroscopy. 3rd ed. Meerut: PragatiPrakashan Educational publishers;2007. p. 1-5,237-314.
8. Tips on Liquid Chromatography, Waters, www.waters.com.
9. Lindsay S. High Performance Liquid Chromatography. 1st ed. New York: WileyInterScience; 1991. p. 4,27,71-9.
10. Fong GW, Lam SK. HPLC in the Pharmaceutical Industry. New York: Marcel Dekker Inc; 1991.p. 47.
11. Ghoush MK. HPLC Methods on Drug Analysis. Berlin: Springer Verlag; 1992. p. 24-6.

12. Meyer VR. Practical High Performance Liquid Chromatography. 2nd ed. New York:Wiley InterScience; 1994. p. 98-105.
13. Lough WJ, Wainer IW. High Performance Liquid Chromatography: FundamentalPrinciples and Practice. Glasgow (UK): Blackie Academic & Professional; 1995. p. 23-8.
14. International Conference on Harmonization. Q2A:Text on Validation of Analytical Procedures. Federal Register;1995. 60(40). p. 11260–2.
15. International Conference on Harmonization. Q2B:Validation of Analytical Procedures:Methodology. Federal Register; 1997. 62(96). p. 27463–7.
16. FDA. Analytical Procedures and Methods Validation:Chemistry, Manufacturing and Controls Documentation.Availability. Federal Register; 2000. 65(169). p. 52776–7.
17. www.fda.gov/cder/guidance/cmc3.pdf
18. United States Pharmacopoeia (USP-NF XXIV). Rockville MD 20852. United StatesPharmacopoeialConvention ; 1985.p. 2149-51.
19. Synder, Lioyd.R, Kirkiand, Joseph.J,Giajch,Joseph.L. Practical HPLC Method Development. 2:686-705.
20. USP 25–NF 20, Validation of Compendial Methods Section (1225) United States Pharmacopeal Convention, Rockville, Maryland, USA; 2002. p. 2256.
21. Shabir GA. Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities Between Validation

Requirements of FDA, the US Pharmacopeia and the ICH. J ChromatogrA2003;987(1-2):57-66.

22. Joachim Ermer, Miller. Method Validation in Pharmaceutical Analysis. Weinheim:Wiley-vchVerlag GmbH & Co; 2005.
23. Goodman & Gilman's. The pharmacological basics of therapeutics. 10th ed. McGraw-Hill, medical publishing house; p. 279-281.
24. The Merk Index, an encyclopedia of chemicals, drugs and biological. 14th ed. NJ;2006.
25. Shanmukhakumar JV, Ramachandran D, Settaluri VS, Lakshmi Lavanya A, ShechinahFelice C. Validation of analytical procedures for determination of Mebendazole benzoate. T. Ph. Res. 2010;4:28-37.
26. AcharjyaSasmitaKumari , SahooSubhasish, Dash KiranKoushik, Annapurna MM. UV-spectroscopic methods for estimation of Mebendazole benzoate in pharmaceutical preparations. Int. J. ChemTech Res. Jan-Mar 2010;2(1):653-9.
27. Khedkaramol, RajendraVivek, KulkarniAlpana, DehghanMohd Hassan, Saifee Maria, LahotiSwaroop. Spectrophotometric method for analysis for Mebendazole benzoate. Int. J. Ph. Sci. Sept-Dec 2009;1(2):307-9.
28. Prithviraj S Yadav, Devprakash, Senthilkumar GP and Mani T Tamizh. Determination of Mebendazole in bulk and its tablet dosage forms by UV spectroscopic method. IJPSR. 2011;Vol. 2(8): 2041-44.
29. Ramzia I El. Bagary, Nashwah G Mohammed and Heba A Nasr. Flourimetric and colorimetric methods for the determination of some antimigraine drugs. J. Chem. Pharm. Res.2011;3(4):304-314.

30. Madukar A, Kannapan N, Ganesh A, Naveen Kumar CH, Mannavalan R. Analytical method development and validation of Mebendazole benzoate tablets by RP-LC. Int. J. PharmTech Res. Oct-Dec 2009;1(4):1704-8.
31. Devprakash, Sumalatha BV, SuhasGurav, Prithviraj S Yadav and SenthilKumar GP. Estimation of Mebendazole benzoate by RP-HPLC method in bulk and dosage form. Journal of Pharmacy Research 2012;5(1),120-123.
32. PunnaVenkateshwarlu and SrikanthGajam. A validated and simplified RP-HPLC of Mebendazole benzoate from bulk drugs. JPBMS 2011;8(05):1-4.
33. Murthy T.E.G.K and Niharika V.L. Reverse phase HPLC method for the estimation of Mebendazole benzoate from formulated oro-dispersable tablets. Pharm Ana &QualAssur 2011.
34. Sachin S Jagatap, Gopu CL, Kakasaheb RM and Mahadev VM. Stability indicating reverse- phase high- performance liquid chromatographic method for the determination of Mebendazole benzoate in bulk and its pharmaceutical formulations. RJPBCS. 2010; vol1(2): 385-395.
35. Ramzia I El. Bagary, Nashwah G Mohammed and Heba A Nasr. Two chromatographic methods for the determination of some antimigraine drugs. Anal Chem Insights 2012; 7:13-21.
36. DevprakashDahiya and PrithvirajYadav. Determination of Mebendazole in bulk and its tablet dosage forms by HPTLC method. Journal of Pharmacy Research 2011;4(6),1788-1790.
37. SyamaSundar B and Suneetha A. Development and validation of HPTLC method

for the estimation of Mebendazole benzoate in bulk and tablets. Indian J Pharm Sci. 2010; vol 72(6): 798-801.

38. Trinath M, Saurabh K Banerjee, Hari Hara Teja D, Bonde CG. Development and validation of spectrophotometric method for simultaneous estimation of Sumatriptan and Naproxen sodium in tablet dosage form. *Der Pharmacia Sinica* 2010;1(1):36-41.
39. BuridiKalyanaRamu, K Raghubabu. Estimation of Albendazole succinate in bulk and formulations by visible spectrophotometry using aromatic aldehydes. IJABPT Jan-Mar 2011;2(1).
40. Shah CR, Suhagia BN, Shah NJ, Shah RR. Development and validation of a HPTLC method for the estimation of Sumatriptan in tablet dosage forms. Indian J Pharm Sci. Nov–Dec 2008;70(6):831–4.
41. PVivekSagar, Dhiraj Kumar, SuddhasattyaDey and HimansuBhusanSamal. Simultaneous estimation of Mebendazole, Albendazole and Zolmitriptan by RP-HPLC method in bulk. JPR 2010;3(12):2930-3.
42. ASuneetha, B SyamaSundar. A validated RP HPLC method for estimation of Albendazole maleate in pharmaceutical dosage form. J. Chin. Chem. Soc. 2010; 57:1067-70.
43. AsadRaza, Tariq Mahmood Ansari, ShahidaBegamNiazi. A novel spectrophotometric method for determination of Zolmitriptan in pharmaceutical formulations. J. Chin. Chem. Soc. 2007;54:1413-7.
44. MadhusudhanareddyInduri, BhargavanRaju M, Rajendra Prasad Y, Pavan Kumar

Reddy K, RajkumarBoddu, SarvaRaidu Ch. A validated RP HPLC method for the quantification of Albendazole in tablet dosage form. *Der PharmaChemica*. 2010; 2(5):351-7.

45. RamakotaiahMogili, KanchanamalaKanala, Balasekhara R Challa, Babu R, Chandu and Chandrasekhar K Bannoth. Determination of Mebendazole in human plasma by liquid chromatography stable isotope dilution electrospray MS-MS for application in bioequivalence study. Chromatographia 2011;74,7-8,585-592.
46. Vishwanathan K, Bartlett MG and Stewart JT. Determination of antimigraine compounds Mebendazole, Albendazole, Naratriptan and Sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom 2000;14(3):168-72.
47. Guo JF, Zhang AJ, Zhao L, Sun XH, Zhao YM and Gao HZ. Determination of Mebendazole in human plasma by liquid chromatographic –electrospray tandem mass spectrometry: application to a pharmacokinetic study. Biomed Chromatogr 2006;20(1):61-6.
48. Qin Yong-ping, Zou Yuan-gao, Liang Mao-zhi and Yu Qin. Determination of Mebendazole in human plasma by RP-HPLC with fluorescence detection. Cinese J. of Phar. Analysis 2006;vol. 26(1): 7-9.
49. Chen J, Jiang X, Jiang W, Mei N, Gao X and Zhang Q. Liquid chromatographic method for the determination of Mebendazole in human plasma. JChromatogr B AnalytTechnol Biomed Life Sci.2004;805(1):169-73.